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CEREALS AS A SOURCE OF VITAMIN B_1 IN HUMAN DIETS

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What are the constituents which *must* be present in their food if animals are to survive? This question has been posed and re-posed by physiology for a century. At times it has been presumed that a complete answer had been secured. Time and again, however, further and more detailed study revealed that earlier complacence was based on crudities of analytical methods, not upon genuine knowledge. Undoubtedly, the fact that animals are commonly observed to live successfully, at least for a time, upon widely differing food supplies contributed to the belief that they could have no very hard, fast, and detailed requirements.

In spite of a generation of intensive and skeptical examination of the question, it is still impossible to make a complete list of all the specific substances which animals require for the maintenance of health and vigor. The list already includes many mineral elements, such as calcium, magnesium, copper, iron, zinc, manganese, iodine, phosphorus, etc., in fact pretty much all minerals of sea water. Several specific amino acids, such as lysine and tryptophane, are recognized as essential, the amino acids appearing in the food as component parts of the proteins. All of these substances are found in the body as constituents of the tissues or body fluids. In addition, there is an already long list of vitamins, by which we merely mean specific organic compounds which are essential in small amounts to proper animal function and which must be supplied in the food, since in general animals have no capacity for synthesizing them. Some of these are fat-soluble substances, such as vitamins A and D; others are water-soluble and wholly different in character. The water-soluble ones include vitamin C (anti-scurvy vitamin) which contains only the elements carbon, hydrogen, and oxygen; vitamin B_2 (riboflavin) and nicotinic acid (anti-pellagra vitamin) contain also nitrogen, while vitamin B_1 (anti-beriberi vitamin) contains, in addition, a fifth element, sulfur.

The vitamins are accordingly very diverse in their chemical nature and exhibit great contrasts in their stability to heat, their distribution and abundance in foods, etc., so that few general statements can be made which are true of them all. That which is common to all of them, aside from their ultimate origin in plants, is a certain, at least apparent, similarity of physiological role. Even this similarity is somewhat vague and, as regards some groups of vitamins, conjectural. However, students of these substances have come in recent years to feel that many, if not all of them, will be found to be parts of enzyme systems, that is, catalytic mechanisms necessary for the conversion of external foodstuff into internal tissue constituents or into vital energy. In some cases, notably those of several of the B vitamins, evidence to this effect is already strong.

More and more it is becoming evident that the vitamins which occur in plant tissues are not present there as accidental components, but that they play roles in the plant world similar to those they play in the animal kingdom. The plant fabricates these substances for its own benefit. Their utilization by animals is a parasitic incident. The vitamins, therefore, constitute another example of the dependence of animals upon plants. As has long been recognized, the latter convert carbon dioxide to carbohydrates by the aid of sunlight and thus furnish an indispensable caloric supply for animals. In this phase the animals degrade the complex organic compounds, notably the carbohydrates, which the plants build up. The two kingdoms are thus in contrast. In another phase, that of the vitamins, the plants carry on many and varied organic syntheses of compounds which are similarly utilized by both plants and animals as mechanisms of cellular metabolism. With respect to these mechanisms, the two kingdoms exhibit kinship rather than contrast.

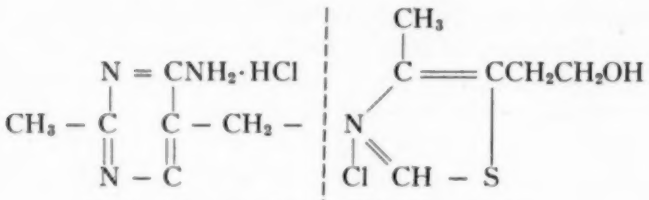
This evidence, that plants and animals utilize in common so many chemical mechanisms, adds a great emphasis to the idea of the essential unity of all forms of life. Both man and the potato on which he sometimes subsists have a common heritage from the earlier and more primitive forms of life from which they sprang. The same mass of evidence also adds to the marvel that life should have developed at all. If such a variety of intricate physiological mechanisms are necessary for the survival of even the simplest forms of life, what countless experiments Nature must have performed before the first viable cell appeared! What incredible combinations of circumstances must have surrounded each successive step of amplification of its initial powers!

In the light of later events it seems likely that the nearly universal

occurrence and physiological role of the vitamins had much to do with delaying our discovery of them. Had these substances been peculiar to a few distinctive plant tissues, as is the case with quinine for example, deficiency diseases would have been so common as to have been recognized as such by the ancients. Almost any combination of ordinarily available foods contains some of all of the vitamins (or their precursors) because the foods, being living tissues, required their presence to exist. So it was only under rather exceptional circumstances that deficiency diseases appeared in human beings. Thus sailors on long cruises, soldiers in beleaguered camps, and arctic explorers at a distance from their base of supplies fell a prey to scurvy because their rations were unusually restricted. Likewise, beriberi became prevalent in the Orient only as increasing population density restricted the people more fully to a rice diet and the advent of factory milling of the rice deprived it more fully of its external coats. In short, primitive man was protected from outright deficiency disease largely because he had a common descent and, in part, a common physiology with the plants which he or his animal prey used as food. That such is the case will become clearer from the account given below of the physiological role of vitamin B_1.

It must be regarded as highly significant that early studies of vitamin B_1 were associated almost wholly with the human disease beriberi. Takaki largely eradicated the disease from the Japanese navy more than fifty years ago by substituting beef and barley for a part of the polished rice of the ration. Thus decorticated grain was condemned by practical experience long before laboratory men began to inquire as to the specific reason. Not until ten years later did Eijkman in Java reproduce the disease experimentally in chickens by feeding them on polished rice. He thus initiated the long series of studies in many lands, which progressively showed that the bran coats of grains contain in very small amounts a substance which is a specific preventive and cure for the disease. Thirty years after Eijkman's work began the pure substance was at last isolated by Jansen and Donath in the same laboratory in Java where their fellow countryman had labored. The amounts obtained were so small that even the elementary composition of the substance was not correctly determined at that time. Seven years later larger yields were obtained by Williams and his associates in America, and the work of determining its structure began.

It turned out to be a unique compound, consisting of a pyrimidine and thiazole having the following formula:



Pyrimidine portion | Thiazole portion

Thiamin Chloride Hydrochloride

Its synthesis followed almost immediately. The whole matter has since been confirmed by British, German, and Japanese workers and commercial production of the substance has already reached hundreds of kilos. The substance has recently been given the name thiamin to identify it as the sulfur-containing vitamin.

No sooner had the synthetic substance become available for experimental work than evidence began to pour in from many sources regarding the nature and extent of its role in living things. First we may mention the discovery of Lohmann and Schuster that thiamin pyrophosphate occurs as such in yeast and is identical with the previously postulated cocarboxylase. This compound is a coenzyme, that is, a component part of an enzyme of yeast which is essential to the production of carbon dioxide from sugar. More specifically the enzyme functions at a particular stage of the fermentation process, involving the conversion of pyruvic acid, $CH_3COCOOH$, to acetaldehyde and carbon dioxide.

Thiamin, either free or in the form of its pyrophosphate, plays a similar, though perhaps not quite identical role, in the metabolism of animal tissues. Peters has long since shown that the brain tissue of beriberi pigeons cannot respire properly in a pyruvic acid medium. On addition of thiamin to the medium the brain tissue promptly consumed oxygen and gave off carbon dioxide more rapidly than prior to the addition. With thiamin present it behaved much more nearly like normal pigeon brain tissue and was able to utilize pyruvic acid, which otherwise accumulated in the tissues as a result of the unfinished metabolism of sugar. The impairment of pyruvic acid utilization in beriberi is not peculiar to brain tissue. Kidney, heart, and liver may show it, and it is fair to assume that it occurs in the cells of all organs in greater or less degree. It is of interest to note that pyruvic acid has since been found in abnormally large amounts in the blood and urine of people suffering from beriberi.

It seems a far cry from yeast cells to human tissues. Actually, they are not as far apart as it would seem, for a great mass of evidence

has accumulated indicating that the metabolism of sugar follows a more or less common pathway in all living things. Thus, many of the long series of steps by which glucose is converted to carbon dioxide and water in the yeast cell, are present as part of the process of contracting muscle or functioning nervous system in animals. There is reason to believe that in all living things the first step in the metabolism of glucose is its conversion into phosphoric ester, and this is followed by a splitting of the six carbon atom chain of the sugar into two phosphorylated fragments, each of three carbon atoms. These fragments lose phosphoric acid and are converted into pyruvic acid, a substance which is therefore to be regarded as a well-nigh universal intermediate in the process of sugar utilization. The course of the process beyond that point is more debatable, but it seems very likely that the availability of thiamin will be of great assistance in solving the problem, because it is at this stage of carbohydrate metabolism that thiamin evidently enters as a component of one or more necessary enzyme systems. Apparently the process can go normally thus far without thiamin, but halts badly at this point.

Since most living things need to metabolize carbohydrate at one stage or another of their life cycles, it is not surprising that a great many organisms have been found to require thiamin for their normal function. Thus, lactic acid bacteria contain it and use it as a part of their life process. This is equally true of staphylococcus, a common pus-forming organism. Many saprophytic plants, that is, plants which live upon the dead remains of other organisms, rather than solely upon inorganic substances, depend upon the thiamin which is present in these organic remains. Therefore, the thiamin requirements of many molds and fungi have been investigated with definite results. Certain fungi have even been proposed as a possible means of determining the amount of thiamin in a food extract or body fluid. Very generally it has been proved to be the case that organisms grow in proportion to the amount of thiamin which is present. Even yeast, which is well known as a rich source of thiamin, will grow much more thriftily in the presence of an ample supply, and will exhibit the power to ferment sugar correspondingly more rapidly. It does appear that yeast has a certain capacity to synthesize thiamin, although I doubt whether the evidence is quite beyond dispute. Be that as it may, it is certainly true that the yeasts which are richest in thiamin are those grown in media which contain a great deal of it. The fact that beer is almost free from thiamin, even though the original wort was rich in it, is evidence of the great avidity with which the yeast cell seizes any available supply of the substance and incorporates it within itself.

As the studies of the effect of thiamin on the growth of a wide

variety of plant tissues has proceeded, the interesting observation has been made that the intermediates in the artificial synthesis of thiamin will sometimes serve as a substitute for the finished product of synthesis. Thus, some plants or plant tissues require the thiazole portion of the vitamin in order to grow, others more conspicuously need the pyrimidine portion, still others require a mixture of the pyrimidine and thiazole portions. Only a few plants have been found which require the complete molecule in order to carry out their normal processes. This observation has been of great aid in making a survey of the synthetic capacity of living things. The survey is, of course, as yet a very preliminary one, but it indicates at once a well-nigh universal need for thiamin in plant tissues and a highly variable capacity to synthesize it. We must suppose that those which require only the thiazole portion are able to synthesize the pyrimidine portion, and *vice versa*. Some can synthesize neither of the two portions, but show an ample capacity to put the two portions together to produce the final product, thiamin. Thus, many plant tissues fail at one stage or another in ability to synthesize a thing which they all need. We know this from experiments which involve the growing of one organism successively after another in the same identical lot of medium. Thus, Phytophthora grows readily in a medium in which Phycomyces has grown, although the former is entirely unable to grow in the medium as freshly prepared.

In the light of what has been said above, a well-nigh universal occurrence of thiamin in all foodstuffs becomes highly significant. A little of it is present in all live things because it is necessary to their life processes. Even polished rice and white flour contain measurable amounts of it. It is also highly significant that very few foodstuffs contain much of it. This we believe is a reflection of the fact that the capacity for the synthesis of this substance is rather limited. Living things, in general, seem able to make very little more of it than they need for their own growth. To what extent this is due to the instability of the substance once it is formed, and to what extent it is due to meagerness of synthesis is unknown, but it does seem very clear that the accumulated supply of Nature is marginal. This is certainly generally true in the animal world, as there is no very extensive storage of the substance in the body. Given a rich supply of the food the excretion in the urine rises correspondingly, and only a few weeks of feeding on a thiamin-poor diet is sufficient to deplete the body of its reserve and bring on beriberi. Accordingly, the appearance of this deficiency disease so early and so universally among animal species is an indication of their spendthrift character in this regard. The speed of onset is roughly proportional to the metabolic rate and is

thus greatest in the smallest animals. These metabolize food rapidly in order to offset loss of body heat from the skin surface.

If, as we have seen, various plants and micro-organisms differ with respect to their capacities for synthesizing the components of thiamin and for effecting the final union of these components, it might also be expected that a given plant would exhibit variations in synthetic capacity at different stages of its life cycle. Indeed, it is only under highly artificial conditions, such as, in purified media, or by the use of excised portions of plants, that their needs for thiamin can be demonstrated. The whole plant growing under natural conditions usually finds thiamin in its surroundings. But if we isolate a properly chosen bit of plant from external sources of thiamin, we find the need existing. This is conspicuously true of the higher plants. The evidence is that the higher plants synthesize the vitamin in their green leaves but store only limited quantities of it there. The leaves constitute the source of supply for the roots and other parts, and their synthetic capacity corresponds roughly to the growth impulse in them. Thus, the shoots of fresh young grasses contain more thiamin than those of old mature plants, which are approaching the end of their season.

This brings us to the particular theme which will be of special interest to the cereal chemist. It is well known, of course, that higher plants store a reserve supply of food in their seeds to enable the young seedling to grow until it has established itself in its new environment, by putting forth roots into the soil and pushing shoots up into the air and sunlight. Some seeds store fats, but in many instances the reserve food supply takes the form of starch. This is conspicuously true of cereals, the seeds of which, by a long process of artificial selection, have been developed to contain a maximum of this component. Their external parts are richer in thiamin than any other plant (or animal) tissue.

Starch, being a carbohydrate, inevitably passes through the glucose stage in its metabolism. For the metabolism of this glucose, we should confidently expect that thiamin would be required. It is, therefore, almost unmistakable that Nature's reason for a high content of thiamin in seeds is that they also have a high content of starch which the germinating plants must metabolize. This has been amply demonstrated for a limited number of plants by their cultivation in artificial media. Excised pea embryos, although they contain a little thiamin, will grow very slightly in a sugar solution. Too large a part of the thiamin has been discarded with the rest of the seed. Add thiamin to the medium and the growth is multiplied several fold. Tomato roots furnish even more striking experimental material. For this purpose allow tomato seeds to germinate in a pure sugar solution. When the

rootlets have formed, cut off their tips, and transfer the tips to a new flask of sugar solution. They stop growing, not because they have been severed from the seed but because they can no longer draw upon it for thiamin. Add synthetic thiamin to the new flask and copious growth of the roots will occur apart from the seed, in proportion, over a wide range, to the amount of thiamin added.

There is here, we confidently believe, a profound bit of philosophy regarding the selection of human food. When one eats eggs or oysters, one takes advantage of everything that the living organism contains. The very fact that the egg or the oyster has grown and developed and is capable of further and independent growth and development is evidence that it contains all that, at least a restricted, life requires. Eat liver, kidney, or pancreas and one gets the products of varied glandular activity. Eat milk and one gets the entire natural food supply of the young. Eat leaves and one is consuming tissue that yesterday, or a week ago, was in the active process of growth and, accordingly, of thiamin synthesis. Eat the whole seed and one assimilates the entire organism which, while it is for the moment at a dormant stage, is still demonstrably capable of a lively development. But, carefully to sift the starch from the other parts of the seed and make the starchy endosperm the chief source of energy for human life, is to fly in the face of Providence.

By fortunate accident there are few foodstuffs which lend themselves so readily to sterilizing purification. One may, as the Eskimos do, eat whale blubber, or one may try out lard or express corn oil for human consumption, but that is not so bad, for the purified foodstuff, being fat, does not require thiamin for its metabolism. Only the producer of refined cane sugar can rival the miller of rice or wheat (the world's two largest cereal crops) in the thoroughness of the devitalizing purification process.

Unquestionably, from a long range business standpoint, the greatest opportunity for the miller, and the sugar refiner as well, is to find a way to meet popular demands without continuing to call upon their customers to disregard Nature's laws, or to make up the deficiencies of the carbohydrate staples by judicious use of other foods. One cannot of course ignore the popular fancy for white bread, nor the concrete fact that whole cereals or their mill products are more subject to spoilage than the whiter forms (in part, for the excellent reason that the latter will scarcely support bacterial or insect life). One cannot wholly ignore the testimony of qualified physicians, that branny roughage is irritating to the intestinal tracts of some people, though that is probably more a matter for the hospital dietician than for the housewife. All these are parts of the large problem which the carbohydrate

industries face, that of making their staple products more nearly the equivalent in nutritive value of the whole seed or the cane stalk, as it was once consumed by primitive man. Whether this is to be done by additions of synthetic materials or by retention of the original nutritive components of the crude foodstuffs is a question for industry to decide. To blink at the scientific facts, which will presently become common knowledge, will be suicidal for the commercial enterprises concerned.

STUDIES ON WHEAT STARCH. I. THE AMYLOPECTIN AND AMYLOSE CONTENT OF VARIOUS WHEAT STARCHES [1]

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Constituents of Starch

Careful analysis of starch reveals that it is not a homogeneous substance, but that noncarbohydrate constituents such as phosphorus, fatty acids, nitrogenous material, and silicon are also present in small amounts in the different kinds of starches.

The presence of phosphorus in starch is generally conceded, but its distribution in the starch granule has been the object of much controversy. Samec (1934) suggested that phosphoric acid in starch is in some manner combined with the nitrogenous substance and united with the polysaccharide in the form of "phytovitellinen." Koets (1935) suggested that the amylopectin fraction of starch is a complex coacervate of amylophosphoric acid and a nitrogenous substance, probably of protein character. Samec and Beniger (1931) reported that at least a portion of the nitrogen in starch occurs in chemical combination. Samec (1934) believes that in some starches the phosphoric acid is present as a complex silicophosphate which in wheat starch is deposited as an insoluble salt, for example, a calcium salt, and in potato starch as a soluble potassium salt. Taylor and Lehrman (1926) found that the fatty acids of corn starch consist of approximately 24% palmitic, 40% oleic, and 36% linoleic, and (1930) that those of wheat starch are about 35% palmitic, 41% oleic, and 24% linoleic.

The starch granule consists chiefly of two carbohydrate constituents. Meyer (1895) introduced the names alpha-amylose for one portion and beta-amylose for another portion of the starch granule.

[1] Paper No. 1655, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Olof E. Stamberg to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1938.

Maquenne (Walton, 1928) used the term amylopectin instead of alpha-amylose, and amylose instead of beta-amylose. Both terminologies are now used in the current literature. Samec and coworkers (Samec and Mayer, 1921; Samec and Waldschmidt-Leitz, 1931) prefer the names amyloamylose and erythroamylose for two starch fractions obtained by electrophoresis, with the erythroamylose migrating to the anode.

Samec and Haerrdtl (1920), Samec and Mayer (1921), Samec (1934), Sherman and Baker (1916), Taylor and Walton (1929), Baldwin (1930), Kavcic (1930), and Stamberg (1936) found practically all the phosphorus in the amylopectin fraction. Other investigators including Karrer and Kraus (1929), Taylor and Iddles (1926), Hirst, Plant, and Wilkinson (1932), and Taylor and Schoch (1933) found that both starch fractions contained about the same amount of phosphorus. Widely different methods of fractionation were used by these investigators and incomplete separation in some instances would lead to such differences in results. Taylor and co-workers found the fatty acids of starch entirely in the amylopectin fraction and considered the presence of fatty acids rather than phosphorus to be the primary difference between amylose and amylopectin.

It is generally agreed upon by various investigators that the amylose is quite soluble in water, has a lower viscosity than amylopectin, and gives a blue iodine color, and that the amylopectin is less soluble, has a higher viscosity, and gives a red-violet color with small amounts of iodine.

Structure of Starch

The presence of a membrane around the starch granule is frequently referred to in the starch literature. If such a membrane exists, it is probably not the amylopectin fraction. The present evidence points to a fairly uniform distribution of amylose and amylopectin throughout the granule, and that the outer portion is in a less hydrated state. Morris (1934) suggested that a membrane of less hydrated or retrograded amylose might exist while the starch granule is yet in the plant. Sande-Bakhuyzen (1925) observed that the surface was the most dehydrated part of the starch granule. Alsberg (1937) stated that "The observations of Lynst-Zwikker, 1921, which the writer has been able to confirm, indicate that the membrane of gelatinized granules is an artifact formed during gelatinization by accumulation of less soluble materials at the periphery of the granule."

Hanson and Katz (1934) found that potato and wheat starches, following a treatment with hydrochloric acid and calcium nitrate, were divided radially and tangentially into small blocks of about 1μ, which

were separated by some other substance. They assumed that the blocks were the amylose and that the amylopectin was the substance interposed between them. The same block structure was observed by Badenhuisen (1937, *a* and *b*) in wheat starches, but he concluded that the blocks appeared only upon gelatinization of the granules. Nikolaieff and Schultz (1933) suggested that the wheat starch granule is made up of two lens-shaped halves held together by radially oriented micelles. Sande-Bakhuyzen (1926) grew wheat under constant artificial illumination. The starch granule did not have the rings characteristic of ordinary wheat starch, and radial needles resembling pyramids with a base of 2–3μ were observed in the granules.

As to the chemical structure of the starch molecules, Hirst, Plant, and Wilkinson (1932) give the well known Haworth model of 24 to 30 glucopyranose units for both amylopectin and amylose, stating that in amylopectin the molecules are denser and more interlocking than in amylose. Staudinger and Husemann (1937) studied the properties of starch and starch derivatives by viscosity, cryoscopic, and diffusion methods. They stated that the Haworth straight chain model of starch does not explain the difference in diffusion constants obtained by starch and cellulose. They suggested that the starch molecule includes a main chain with some side chains linked through their aldehyde groups to the main chain, thus producing fewer free reducing groups. Caldwell and Hixon (1938) concluded after a series of periodic acid oxidation studies of several starches and dextrins that the average chain length of the starch molecule must be considerably greater than the 25 glucopyranose units estimated by Haworth.

Myrbäck and Ahlborg (1937) were in agreement with Staudinger and Husemann that the starch molecule is probably a branched chain system, and Myrbäck (1937) suggested that the reason for the incomplete hydrolysis of starch by beta-amylase might be found in certain anomalies in the starch molecule such as branch chains, or phosphorus and fatty acids in ester linkages. He suggested that further studies of the limit dextrins resulting from enzyme hydrolysis of starch will probably show linkages of glucose units other than the typical maltose linkage. In this connection it is interesting to note that in the diagram by Staudinger and Husemann with the branched chain linked through the number 6 carbon of the main chain glucose unit, there is a possibility of obtaining gentiobiose (glucose, 6, β-glucoside) from starch. Berlin (1926, *a* and *b*) actually reported the isolation of gentiobiose from the residue commercially known as "hydrol" obtained in the manufacturing of *d*-glucose from corn starch. He also demonstrated that gentiobiose is identical with Fisher's (1896) isomaltose produced by the action of strong hydrochloric acid on glucose. Since acid

hydrolysis is used in commercial preparation of glucose from starch and since gentiobiose is identical with Fisher's isomaltose, the question remains whether the gentiobiose obtained by Berlin was a residue from the starch or a product of synthesis. Further studies on the dextrins from starch with its probable "anomalies" will undoubtedly furnish valuable clues as to the structure of the starch molecule.

Experimental

Preparation of the starches.—Five different wheats were used for the preparation of starch and Table I gives their source, crop year, and bushel weight. A Thatcher wheat of normal and one of low bushel weight were included. The wheat samples were milled in the experimental mill and flours of about 80% extraction were prepared.

In an effort to retard the diastatic action, small doughs were mixed at 5° C., using the flour and a 2% sodium chloride solution at the same temperature. After 20 minutes the starch was washed free from most of the gluten over a fine sieve. The starch was recovered by means of

TABLE I

Wheat	Source	Crop year	Bushel weight
Little Club (*T. compactum*)	Pullman, Wash.	1936	62 lbs.
Federation (*T. vulgare*)	Pullman, Wash.	1936	62 lbs.
Durum (*T. durum*)	Minnesota	1936	59 lbs.
Thatcher (*T. vulgare*)	Minnesota	1936	56 lbs.
Thatcher (*T. vulgare*)	Minnesota	1935	48 lbs.

a cup centrifuge, resuspended in distilled water at 5°–8° C., and precipitated again in the centrifuge. This was repeated twice at the same temperature, and three to five times at room temperature. The protein content of the flours, the starch yield, and the nitrogen content of the starches are shown in Table II. A sample of commercial wheat starch was also included.

Separation of small and large granules.—Alsberg (1937) separated the small and the large starch granules by using an air classifier. The principle of this method is that in a constant flow of air the smaller granules will rise to a higher level than the larger. This method was tried, but the difficulties encountered in dispersing the granules and the larger quantities of starch required made it unsuitable.

For this study the small and the large granules of starch Thatcher 56 and commercial starch were separated by means of a specially built sedimentation column. The sedimentation column employed was 15 cm. in diameter and 120 cm. high with three dampers which could be turned, thus dividing the column into four equal parts. The lower

TABLE II

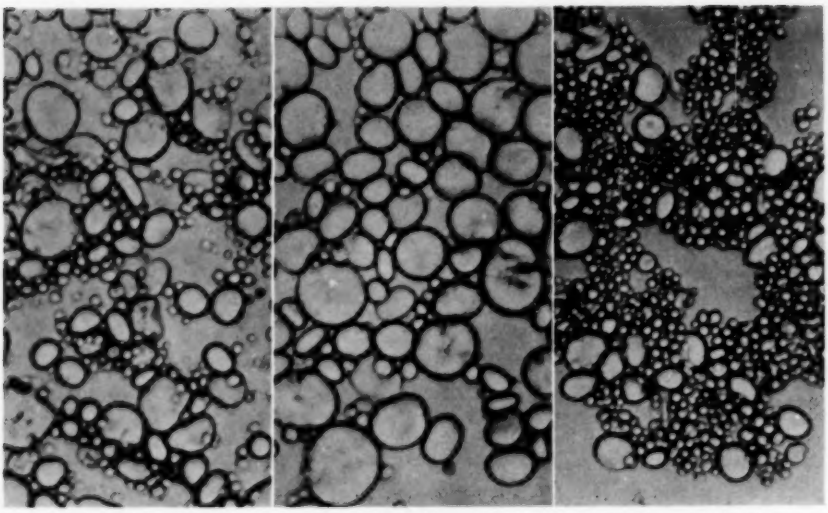
PROTEIN CONTENT OF THE FLOURS—YIELD AND NITROGEN CONTENT OF THE STARCHES

Material	Protein content of flour (N×5.7), 15% moisture basis	Yield of starch, air-dry basis	Nitrogen content of starch, moisture-free basis
	%	%	%
Little Club	6.37	59.5	0.048
Federation	8.70	56.8	0.035
Durum	15.81	44.0	0.045
Thatcher, 56 lbs. per bushel	13.99	52.7	0.056
Thatcher, 48 lbs. per bushel	16.17	38.7	0.036
Commercial	—	—	0.054
Thatcher, 56 lbs., small granules	—	—	0.056
Thatcher, 56 lbs., large granules	—	—	0.042
Commercial, small granules	—	—	0.051
Commercial, large granules	—	—	0.052

parts were filled with distilled water and into the upper part was added a suspension of 100 g. of starch. The dampers were opened and the sedimentation was allowed to proceed for almost an hour when the dampers were closed and each starch fraction was removed through spigots and then recovered by means of the cup centrifuge. Some 15 to 20 refractionations were necessary for each 100 g. of starch before a fairly pure fraction of small and one of large granules were obtained. No attempt was made to make quantitative separations. Some toluol was used in the water to prevent bacterial action. The nitrogen contents of the two final starch fractions are included in Table II. The photomicrographs in Figure 1 show the original Thatcher 56 starch and the small and the large granule fractions. The majority of the large granules were from 20 to 35μ in diameter and the small granules from 3 to 6μ in diameter. The fractions from commercial starch were similar to those shown in the photomicrographs.

Grinding of the starch.—The starch granules must be thoroughly disintegrated before they can be successfully separated into amylopectin and amylose by the electrophoretic method. Taylor and Keresztesy (1936) showed that the amount of amylopectin obtained from corn starch depended upon the length of grinding previous to the separation by the electrophoretic method. Using a ball mill, they found that 168, 336, 672, and 1,344 hours of grinding gave 18.6, 14.5, 8.1 and 6.2% of amylopectin, respectively. This indicates that the amounts of amylopectin and amylose thus separated are relative rather than absolute, and the percentages depend upon the degree of disintegration of the granules.

A rod mill was found to have a much more rapid grinding action than a ball mill. A one-gallon ball-mill jar with thirty steel rods



Original starch Large-granule fraction Small-granule fraction

Fig. 1. Photomicrographs of Thatcher 56 wheat starch.

13 cm. long and 2 cm. in diameter was rotated at the rate of 14.8 r.p.m. All samples were ground for 84 hours, which according to microscopic observations was sufficient to thoroughly disintegrate all the starch granules. Samples of 100 g. each were ground except for the small- and large-granule fractions, of which only 75 g. samples were available.

Separation of the amyloses.—The electrodialyzing apparatus used in the separation of the amyloses was a slightly modified type of that described by Taylor and Iddles (1926). It is shown by the diagram in Figure 2. The bottoms of jars A and B were collodion membranes deposited on cheese cloth. Leaking membranes must be guarded against. Hard carbon electrodes were used, and a direct current potential of 220 volts.

Exactly 1,000 c.c. of 1.1–1.3% boiled solution of the pulverized starch was introduced quantitatively into jar B. The electrodes were placed in position and the current was turned on. After three days the migration was complete and the amylopectin formed as a slimy layer at the anode membrane on the bottom of jar B. The supernatant liquid was almost clear. It was carefully siphoned off into a liter volumetric flask and made up to volume by distilled water. A 50 c.c. aliquot was dried at 110° and used to determine the amount of amylose removed. The amylopectin layer was redispersed in a liter of hot water, and upon cooling was subjected to another electrophoretic separation. This was repeated about six additional times to remove all of the amylose as determined by the absence of weighable solids

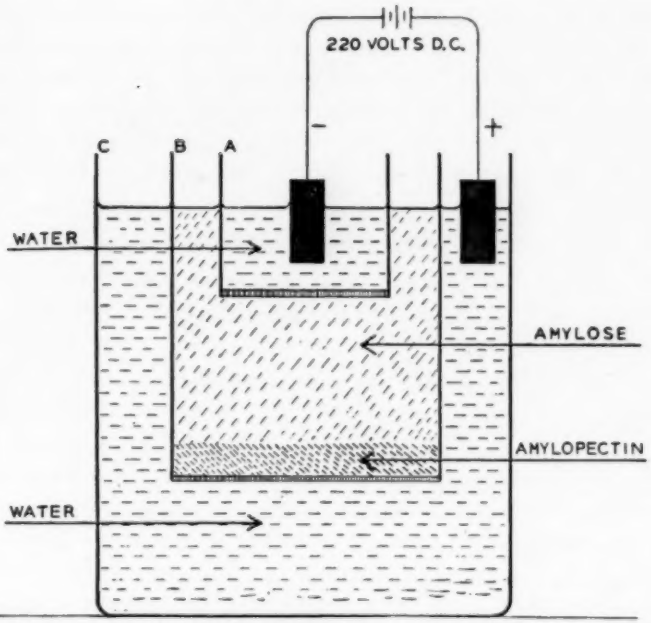


Fig. 2. Diagram of the electrodialyzing apparatus. Necessary clamps and holders are not shown. Dimensions of the cells: A, diam. 10 cm., ht. 10 cm.; B, diam. 14 cm., ht. 19 cm.; C, diam. 26 cm., ht. 30 cm. Collodion membranes on cheese cloth.

in the aliquot from the supernatant liquid. The percentage of amylopectin was determined by the difference between the amount of starch used and the amylose removed. A few drops of toluol were always added to the starch solutions to prevent bacterial action. The percentages of amylopectin from two separations of each starch sample and the average values are given in Table III.

TABLE III

RESULTS ON AMYLOPECTIN AND PHOSPHORUS DETERMINATIONS

Starch used	Grinding of starch in rod mill		Amylopectin			Phosphorus in			Total phosphorus of starch in the amylopectin
	Hours	Amount	1	2	Av.	Starch	Amylose	Amylopectin	
		g.	%	%	%	%	%	%	%
Little Club	84	100	15.15	14.99	15.07	0.050	0.002	0.238	71.8
Federation	84	100	16.92	15.02	15.97	0.059	0.002	0.387	102.5
Durum	84	100	16.26	16.10	16.18	0.070	0.003	0.404	93.3
Commercial	84	100	17.13	17.38	17.26	0.061	0.001	0.261	73.8
Thatcher, 56 lbs. per bu.	84	100	15.36	14.31	14.84	0.068	0.001	0.354	77.2
Thatcher, 48 lbs. per bu.	84	100	15.21	16.00	15.60	0.073	0.004	0.437	93.2
Commercial, small granules	84	75	15.79	14.85	15.32	0.057	0.001	0.293	78.8
Commercial, large granules	84	75	14.45	15.08	14.76	0.055	0.004	0.344	92.3
Thatcher, 56 lbs., small granules	84	75	13.32	12.58	12.95	0.068	0.007	0.483	92.0
Thatcher, 56 lbs., large granules	84	75	11.82	11.97	11.90	0.061	0.007	0.436	85.1

Phosphorus determinations.—Since the phosphorus content of amylopectin and amylose and large- and small-size starch granules have been problems of interest, phosphorus determinations were made by the method described by Morris, Nelson, and Palmer (1931). The results are shown in Table III, and also the calculated percentages of the total phosphorus of the starch recovered in the amylopectin fraction.

Discussion of Data

In view of the observations of Taylor and Keresztesy (1936) that the amount of amylopectin obtained is only relative and depends on the degree of disintegration of the granules, the values obtained must be considered as relative and not absolute until the two starch fractions have been more thoroughly defined. The percentages of amylopectin obtained from the six kinds of wheat starches appear to be about the same, and the small differences are probably not significant and are well within the limits of accuracy of the method.

The percentages of amylopectin recovered from the small and large granule fractions indicate that there is probably no difference due to granule size. The separation of the large and the small granules was sufficiently complete (Fig. 1) so that any differences due to granule size would be expected to be quite large. The amylopectin contents of the Thatcher 56 small and large granules were lower than those of other samples, but the higher phosphorus content of the amylose fractions indicates that the fractionation was probably incomplete and that some amylopectin was collected with the amylose fraction. These results are in agreement with the theory that amylopectin is distributed quite uniformly throughout the granule; otherwise, if the outer membrane were amylopectin, the percentage for the small granule would be much larger because of the significantly greater surface area of the small granules.

Grüss (1932) separated the large and the small granules from barley starch by the sedimentation method. Starch pastes were then subjected to a continuous filtration at 60° C. through a porous porcelain filter. The starch in the filtrate was precipitated by an excess of tannin and the precipitate was dried and used as the value for the amylose. By this method, he found 12.8% amylopectin in the large granules and 34.8% in the small granules. The membranes of the gelatinized starch granules probably remained in the filter—hence the larger value for the small granules with the greater amount of membrane.

The phosphorus contents of the six kinds of starches were much in the range found by many previous workers. Practically all the

phosphorus was found in the amylopectin fractions, with only traces remaining in the amylose fractions.

The phosphorus content of the small and the large granules appeared to be approximately the same, and the small differences are not significant in view of the relative completeness of separation of the large and the small granules. This agrees with Alsberg (1937), who separated different sizes of granules from tapioca starch and found that the fractions had much the same phosphorus content. It is further in agreement with Taylor and Schoch (1933) who found that the phosphorus is distributed quite uniformly throughout the granule rather than at the surface. Fernbach (1904) found the small granules from potato starch to be higher in phosphorus than the large granules from the same sample however, and Kavcic (1930) also found that in potato starches there was a tendency for the phosphorus to increase as the granule size decreased.

Summary

Wheat starches from Little Club, Federation, Thatcher of 48 lbs. bushel weight, Thatcher of 56 lbs. bushel weight, and a durum wheat were prepared. These starches and also a commercial wheat-starch sample were finely pulverized in a rod mill, and subsequent fractionations by the electrophoretic method indicated that these starches contained practically the same relative amounts of amylopectin of from 15 to 17%.

Small and large wheat-starch granules separated from the same sample by a sedimentation process were found to contain about the same relative amounts of amylopectin as determined by the electrophoretic method.

There was no significant difference in phosphorus content of the small and the large granules separated from the same samples of wheat starch.

The amylopectin fraction separated by the electrophoretic method contained almost all of the phosphorus of the starch, and only a trace of phosphorus was found in the amylose.

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STUDIES ON WHEAT STARCH. II. THE ACTION OF AMYLASES ON RAW WHEAT STARCHES[1]

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Several factors are probably involved in effecting the differences in diastatic activity of flours in either suspensions or doughs. Among these factors might be (1) inherent differences in the starches from different wheat varieties, (2) effects of soil or climatic conditions during the growth of the grain, (3) size distribution of the starch granules, (4) readily available material such as broken starch granules, (5) types or (6) amount of enzymes present, (7) effects due to harvesting conditions including exposure of the grain in the field, and (8) storage conditions and processes such as steeping and bleaching during milling. Several or all of these factors are probably responsible for the variability in so called "diastatic activity" of flours, which ultimately involves the action of the amylolytic enzymes on the starch substrate.

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Many of the factors mentioned have been investigated. Spaeth (1915) concluded that weak flours have more small starch granules and that the quantity depends upon soil and climate conditions. Buchanan and Naudain (1923) found, however, that strong flours have the largest percentage of small granules, and that the average granule size is most important. Grewe and Bailey (1927) concluded that there is no correlation between granule size and the baking strength and diastatic activity of flours. Karacsonyi and Bailey (1930) and Malloch (1929) showed that overgrinding of flours results in increased diastatic activity. Ivanov, Kurgatnikov, and Kirsanova (1937) found that starch from peas grown in a hot dry climate was hydrolyzed much more slowly than starch from peas grown in a moist climate. Mangels (1926) found raw durum starch more easily attacked by diastase than raw spring wheat starch, and he later (1932) observed that starch from bleached flour was more easily hydrolyzed by taka-diastase than starch from unbleached flour. Sandstedt, Blish, Mecham, and Bode (1937) concluded that with the possible exception of durum wheat flour the unruptured granules of various flours are for all practical purposes identical in their resistance to attack by enzymes.

Although alpha-amylase has generally been found to increase the diastatic activity of flours and the action on raw wheat starches, Blish, Sandstedt, and Mecham (1937) stated that normal wheat flour contains an enzyme factor capable of accelerating the saccharification of raw starch, but that the most active preparation of this raw starch factor can be obtained from malted wheat flour. They also described a non-enzymic inhibitor and a non-enzymic "activator" of the raw starch factor. The latter was thought to function only by counteracting the inhibitor.

Experimental

Preparation of enzymes.—Two preparations of alpha-amylase and two of beta-amylase were made from Thatcher wheat. The beta-amylase preparations were from normal wheat. For the alpha-amylase preparations the wheat was steeped for two days at about 16° C. and then allowed to germinate at 20° C. for three days, after which it was dried at 25° C. One method used in preparing the enzymes was that described by Klinkenberg (1932) by which alpha-amylase is precipitated by 60% alcohol and beta-amylase by 80% alcohol from the respective extracts. The second method was a combination of Ohlsson's (1926) and Klinkenberg's methods. According to Ohlsson the alpha-amylase extract is heated to 70° C. for 15 minutes, thus inactivating most of the beta-amylase present, and the

beta-amylase extract is acidified with hydrochloric acid to pH 3.3 for 15 minutes at 0° C., a treatment which inactivates most of the alpha-amylase. The method employed was to treat the extracts according to Ohlsson's technique and then precipitate the enzymes by Klinkenberg's method. The enzyme preparations made by Klinkenberg's method will be referred to as -1 and those prepared by the combined procedures of Ohlsson and Klinkenberg as -2. A commercial diastase preparation (Merck) was also used and will be referred to as malt diastase.

Action of enzymes on soluble starch.—Before using the enzyme preparations for raw-starch hydrolysis some tests were carried out with Lintner soluble-starch (Merck) solutions as substrates in order to characterize the preparations as to their relative dextrinizing and saccharifying activities.

Wohlgemuth (1908) tests for the dextrinizing action were made by using 3 c.c. of 2% boiled soluble-starch solution buffered at pH 5.1 and 3 c.c. of water and enzyme solution, with hydrolysis for one hour at 40° C. The values as expressed in Table I are in terms of milligrams

TABLE I

RESULTS OF WOHLGEMUTH, CALDWELL-HILDEBRAND, AND SACCHARIFYING TESTS OF THE ENZYME PREPARATIONS, WITH SOLUBLE STARCH AS SUBSTRATE

Enzyme	Wohlgemuth value, mgs. starch per mg. enzyme	Erythro-R value, % hydrolysis	Starch dextrinized but not precipitated in 55% alcohol	Percent hydrolysis, from reducing-sugar determinations (as maltose)		
				In 5 hrs.	In 12 hrs.	In 28 hrs.
	mgs.	%	%	%	%	%
Alpha-amylase-2	214	15.3	64.4	49.5	49.5	49.5
Alpha-amylase-1	171	37.8	46.4	80.0	84.5	89.0
Malt diastase	100	44.4	40.4	78.1	84.5	87.1
Beta-amylase-1	<1	—	7.4	61.0	61.0	63.7
Beta-amylase-2	<1	—	3.7	31.4	52.0	61.0

of starch hydrolyzed to the red-violet (erythro) end point per milligram of enzyme preparation. The reducing values at the end points (the erythro-R values) in terms of percent hydrolysis determined as maltose are also given in Table I.

The dextrinizing activity of the enzymes was determined according to the method described by Caldwell and Hildebrand (1935). A 25 c.c. portion of 2% soluble-starch paste (500 mg.) buffered at pH 5.1 was hydrolyzed for 10 minutes at 30° C. with 10 mg. of enzyme. Ethyl alcohol was then added to give a concentration of 55% by volume. The precipitate was first washed with 55% alcohol and then with 95% alcohol, after which it was dried at 105° C. and weighed.

The percentage of starch dextrinized and not precipitable in 55% alcohol is given for each enzyme preparation in Table I. A higher value indicates higher dextrinizing activity of the enzyme preparation.

The saccharifying activity of the enzyme preparations was compared by using 25 milligrams of enzyme in 100 c.c. of 1% soluble-starch solution buffered at pH 5.1 with hydrolysis at 30° C. Reducing-sugar determinations (calculated as maltose) were made by the ferricyanide reduction method (Blish and Sandstedt, 1933) and Table I gives the percent hydrolysis of the starch after 5, 12, and 28 hours, respectively.

Wijsman (Klinkenberg, 1932) tests were made by placing drops of enzyme solution on a sterile gelatin and starch medium in petri dishes

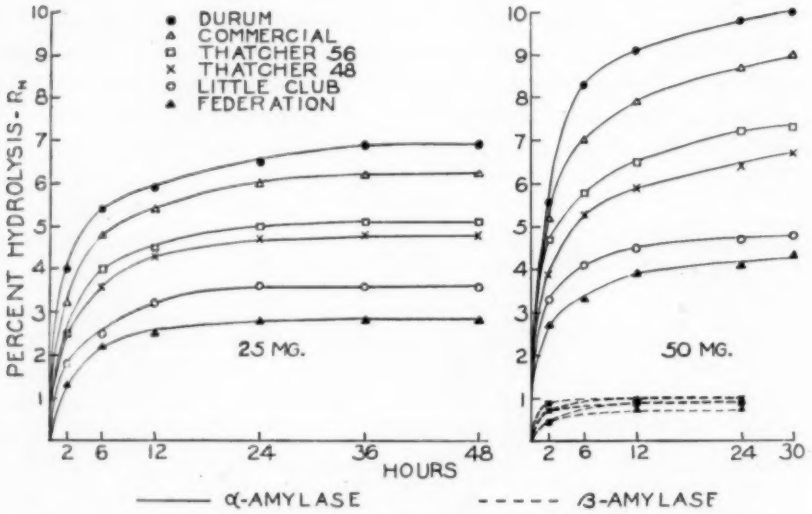

Fig. 1. Action of 25 and 50 mg. of alpha-2, and 50 mg. of beta-2 on raw wheat starches.

which were incubated for four days at 5° C. and then stained with iodine. A colorless spot indicates alpha-amylase and a violet spot beta-amylase, while mixtures of the enzymes produce a colorless spot surrounded by a violet ring. The results indicated that malt diastase and alpha-amylase-1 were mixtures of the two enzymes, and that alpha-2, beta-1, and beta-2 were fairly pure preparations of their respective enzymes.

Action of the enzymes on raw wheat starches.—With the various wheat starches described in Part I available, namely from durum, Thatcher of 48 and 56 pounds weight, Little Club, Federation, and a commercial wheat starch, it was of interest to study the action of the amylases on the raw starches. Small starch granules about 3 to 6μ

in diameter and large starch granules about 20 to 35μ in diameter, separated from Thatcher 56 and from the commercial wheat starch, were also used as substrates. Details as to the method used in separating the granules are given in Part I, which also includes a photomicrograph of the small and the large granules from Thatcher 56. All hydrolysates were 100 c.c. of 1% starch suspensions buffered with citrate solution at pH 5.1 and the temperature was maintained at 30° C. in all cases. Stoppered flasks were used and a drop of toluol was added to prevent bacterial action. A mechanical shaking device was used to keep the starch in suspension. The rate of hydrolysis was followed by determining the reducing value computed as maltose and expressed as percent hydrolysis of the starch present.

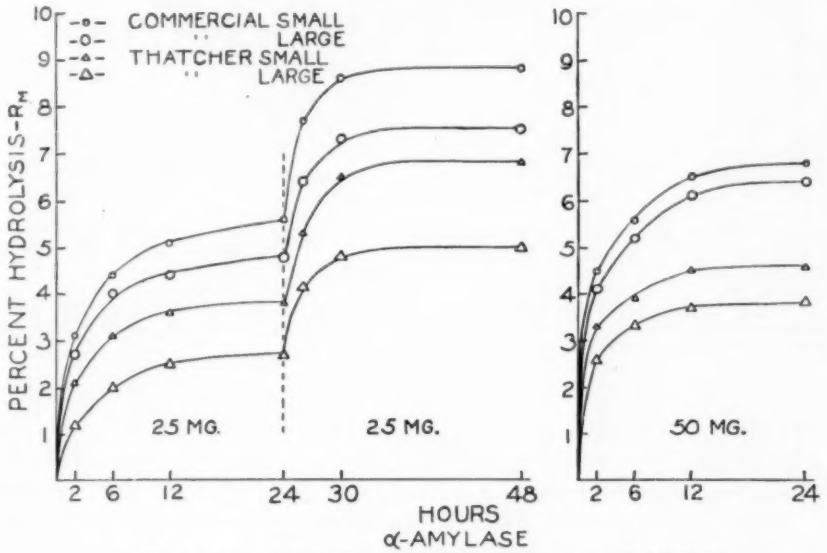

Fig. 2. Action of alpha-2 on the small- and the large-granule fractions.

In the first series of tests 25 mg. and 50 mg. of alpha-2 and 50 mg. of beta-2 were allowed to act upon the raw starches. The hydrolysis was continued for 48 hours when 25 mg. were used, and for 30 hours when 50 mg. were used. The progress of the hydrolysis is shown graphically in Figure 1.

The action of alpha-2 on the small- and the large-granule fractions from Thatcher 56 and commercial starches is shown in Figure 2. In one case two 25-mg. portions were added, and in the other case 50 mg. were added directly.

Since the various starches exhibited differences in their susceptibility to the action of alpha-amylase, as shown by Figure 1, it was of interest to ascertain if the same relative susceptibility remained after

the starch granules had been thoroughly disintegrated. For this purpose, starch samples were used which had been pulverized in the rod mill by grinding for 84 hours as described in Part I. The amount of enzyme used was 25 mg. of alpha-2 and 50 mg. of beta-2. The graphs in Figure 3 show the results of 24 hours of hydrolysis. The action of beta-1 was found to be practically the same as that of beta-2, and only about 1.1% of the raw starches was hydrolyzed during 24 hours.

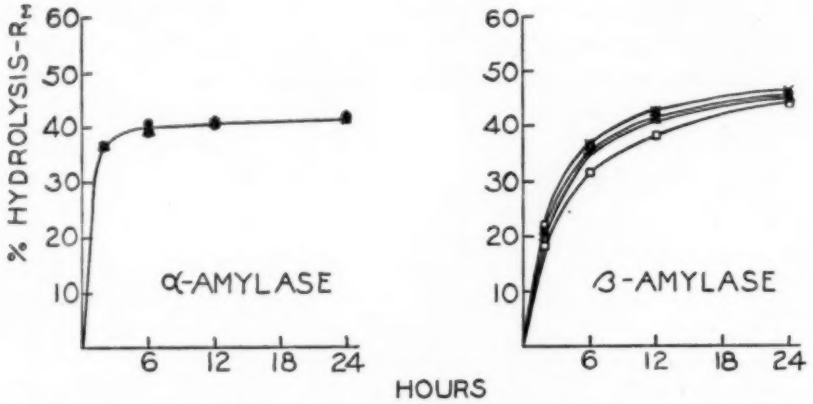

Fig. 3. Action of alpha-2 and beta-2 on the pulverized raw wheat starches. In the case of alpha-amylase, all six starches gave practically the same results and hence only one line was drawn while with beta-amylase a slight variation was noticed.

The hydrolysis of the raw starches by alpha-1, malt diastase, and alpha-2 was then compared. Durum, Federation, and Thatcher 56 starches were used as substrates. The amount of enzyme used was 50 mg. and the hydrolysis continued for 24 hours. In the instance of alpha-1 an additional amount of enzyme equivalent to 50 mg. was added after 24 hours and the hydrolysis continued up to 36 hours. The data obtained are shown by the graphs in Figure 4.

It is evident from Figure 4 that alpha-1 was more active on the raw starches than alpha-2, although the data in Table I show that alpha-2 was the more active dextrinizing enzyme. This suggests the possibility that the greater action of alpha-1 on the raw starches was due to the presence of appreciable amounts of beta-amylase. To study this possible effect of mixtures of the amylases on the raw starches a series of tests was made with alpha-2 and with beta-2 alone and in various combinations. The substrate was raw Thatcher 56 wheat starch. The data presented by the graphs in Figure 5 show the results of these tests and also rate of hydrolysis of alpha-1, so that a direct comparison can conveniently be made.

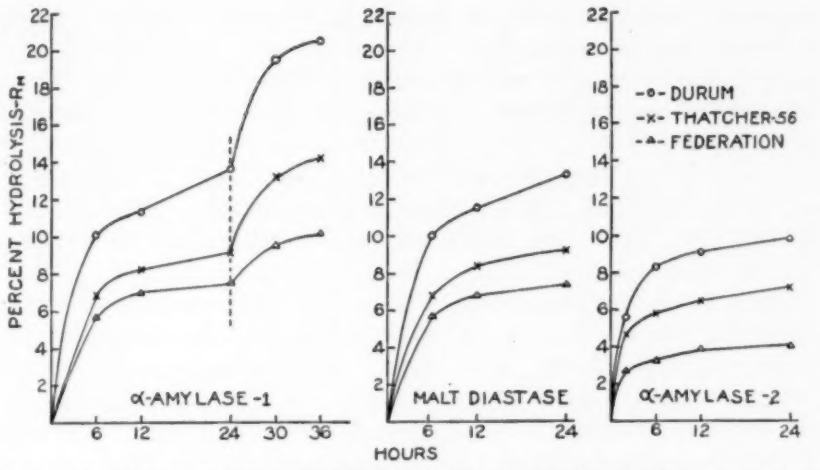

Fig. 4. Action of 50 mg. of alpha-1, malt diastase, and alpha-2 on raw wheat starches. An equivalent amount of alpha-1 was added after 24 hours.

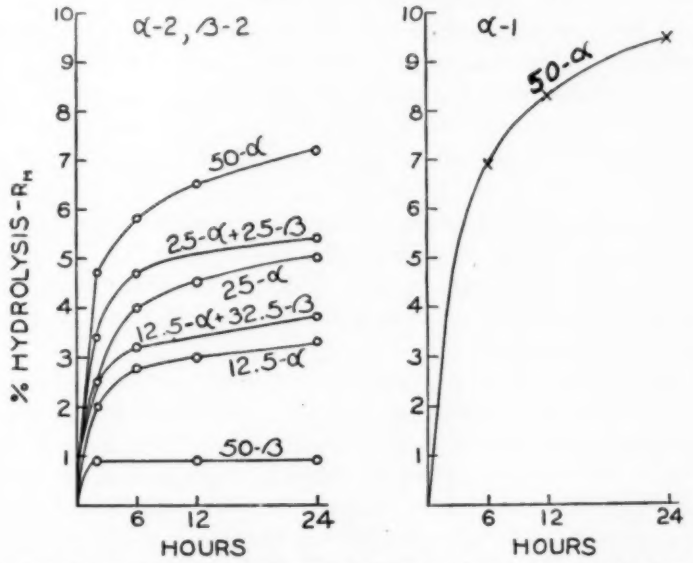

Fig. 5. Action of alpha-2 and beta-2 and various mixtures of them as compared to alpha-1. The milligrams of each enzyme are indicated on the graphs.

Discussion

The results indicate that beta-amylase will not hydrolyze raw starch to a great extent. Only about 1% of the starch was hydrolyzed by the beta-amylase preparations.

The action of the alpha-amylase preparation indicates differences in enzyme susceptibility of the raw starches as shown by Figures 1 and 4. If the observed differences were due to varying amounts of

readily available material such as broken granules, the progress curves with beta-amylase should show such differences, since beta-amylase readily attacks the pulverized granules as shown in Figure 3. Thus it appears quite probable that differences in alpha-amylase susceptibility really existed. The durum starch was most easily hydrolyzed, which is in agreement with Sandstedt, Blish, Mecham, and Bode (1937) and Mangels (1926, 1932). Next were commercial, Thatcher 56, Thatcher 48, Little Club, and Federation in order of increasing resistance to hydrolysis.

The small granules showed slightly less resistance to hydrolysis than the large granules. The fractions from commercial starch were hydrolyzed more readily than the fractions from Thatcher 56, which is in agreement with the original starches as shown in Figure 1. Neither the small nor the large granule fractions were hydrolyzed as readily as their respective original starch samples, suggesting that the additional water treatment necessary to separate the large and the small granules may have removed the more labile material.

Since the small granules were more easily hydrolyzed than the large granules, it seems possible that the observed differences in the enzyme susceptibility of the six starch samples might be due to granule-size variations. A careful analysis of the graphs in Figures 1 and 2 shows that the differences in enzyme susceptibility of the six starch samples were much greater than those of the small and the large granules, and hence it is reasonable to conclude that the differences were not due to the granule-size distribution. This appears to be in agreement with Grewe and Bailey (1927) that there was no correlation between starch-granule size and diastatic activity of flours.

Figure 3 shows that the raw-starch granules which had been pulverized in the rod mill were as easily hydrolyzed by both alpha- and beta-amylase as boiled soluble-starch solutions. Taylor and Kereszttesy (1936) observed that dry grinding or Lintner acid treatment of corn starch produced similar changes when the alkali lability was used as a criterion. Alsberg and Griffing (1925), and Karacsonyi and Bailey (1930) noticed that overgrinding of flour resulted in substantial increase in the diastatic activity.

The enzyme hydrolysis of the ground raw starches further indicates that after the granule structure has been thoroughly broken down the various starches show practically no difference in the rate of enzyme hydrolysis. Thus it appears that the observed variations in enzyme susceptibility of the raw starch granules are due primarily to differences in the granule structure. Probably the porosity of the granule is a factor. Lynst-Zwikker (1921) studied the action of the

amylases on potato and wheat starches and suggested that the amylophosphoric ester in potato starch is combined with potassium and in wheat starch with calcium, and that the calcium ion makes the wheat starch more permeable for the amylases and hence contributes to the greater enzyme susceptibility of wheat starch as compared to potato starch. Samec (1934) also suggested that calcium ions are present in wheat starch and potassium ions in potato starch, but he did not discuss this in relation to enzyme susceptibility.

The conclusion reached in Part I, that there was no significant difference in the relative amounts of amylopectin in the various wheat starches, indicates that the amylopectin content is not the factor involved in the observed variability in resistance to enzyme hydrolysis of the wheat starches. Lynst-Zwikker (1921) reached the same conclusions. On the contrary, Weichsel (1936) concluded that tuber starches such as potato starch differ from wheat starch by having a higher amylopectin content and that this is responsible for the higher resistance of potato starch to enzyme action. She determined the amylopectin content by washing and centrifuging triturated starch and called the insoluble portion amylopectin. Most investigators, however, find less amylopectin in potato starch. Taylor and Iddles (1926) obtained 1.8% from potato starch and Taylor and Walton (1929), using the same electrophoretic technique, reported 25.5% in wheat starch.

The phosphorus content of the various starches is given in Part I, and there appears to be no correlation between the phosphorus content of the wheat starches and their enzyme susceptibility in the raw state.

It seems possible that the small differences exhibited by various types of raw wheat starches are due mainly to morphological differences of the granules as a result of inherent genetic qualities and to environmental factors during growth of the grain.

The actions of alpha-1 and malt diastase on the raw wheat starches were very similar, and both enzymes hydrolyzed raw starch to a greater extent than alpha-2, which was the most active dextrinizing enzyme according to the data in Table I. The results obtained by the various amylase mixtures shown in Figure 5 indicate that the more vigorous action of alpha-1 (and malt diastase) on the raw starches was probably not due to the presence of beta-amylase in appreciable amounts. Although alpha-2 was the most active dextrinizing enzyme with soluble starch as substrate, this enzyme preparation probably contained less of the "raw starch factor" described by Blish, Sandstedt and Mecham (1937).

Figure 4 shows that, upon the addition of a second portion of alpha-1 after 24 hours of hydrolysis, the same relative susceptibility

of the starches was observed. The durum starch was most easily hydrolyzed by both portions of enzyme, followed by Thatcher 56, with Federation starch being most resistant to hydrolysis. This is noticed also by the second portion of enzyme added to the small and the large granule fractions as shown in Figure 2. This indicates that the starch granules are quite uniform in structure throughout.

Summary

Two alpha-amylase preparations were made from germinated wheat, the first by direct alcohol precipitation and the second by a heat treatment of the extract followed by alcohol precipitation; they are referred to as alpha-1 and alpha-2 respectively. Two beta-amylase preparations were made from normal wheat, the first by direct alcohol precipitation, the second by an acid treatment of the extract followed by alcohol precipitation, and are referred to as beta-1 and beta-2, respectively. A commercial malt diastase preparation was also used. These enzyme preparations were first compared by several methods as to their relative dextrinizing and saccharifying activity with soluble starch solutions as substrates.

Beta-amylase hydrolyzed only about 1% of the raw wheat starch granules. Alpha-amylase hydrolyzed raw wheat starches to a greater extent, about 4 to 10% with the amounts of enzymes used, and differences in alpha-amylase susceptibility of the raw starches were noticed. Durum starch was most readily hydrolyzed, followed by the commercial wheat starch, Thatcher 56, Thatcher 48, and Little Club, while Federation starch was most resistant to hydrolysis.

Alpha-amylase hydrolyzed the small granules more readily than large granules in the raw state, but the differences were smaller than those observed for the various starch samples. Hence it was concluded that the differences in enzyme susceptibility of the raw starches were not due to granule size. Neither did the differences appear to be due to more or less easily hydrolyzed material such as broken granules.

Raw wheat starches which had been finely pulverized by grinding for 84 hours in a rod mill were easily hydrolyzed by both beta- and alpha-amylase and with no significant degree of difference in the various starch samples. Such pulverized granules in the raw state were about as easily hydrolyzed as soluble starch paste.

There was no correlation between the phosphorus content of the wheat starches, as given in Table III, Part I, and the enzyme susceptibility of the raw starches.

It was suggested that the small differences exhibited by various types of raw wheat starches are due mainly to morphological differences of the granules.

Alpha-amylase-2, which effected the greatest dextrinization of soluble-starch solutions, was less active on raw wheat starches than alpha-amylase-1 and malt diastase. This was apparently not due to the presence of beta-amylase in the last two enzyme preparations, but it is suggested that possibly alpha-amylase-2 contained less of the "raw starch factor" than the other enzyme preparations.

The rate of hydrolysis of the raw wheat starch granules after successive increments of enzyme were added indicated that the same degree of resistance was present throughout the granule.

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STUDIES ON WHEAT STARCH. III. THE ACTION OF AMYLASES ON WHEAT AMYLOPECTIN AND AMYLOSE [1]

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Many investigators have found that a solution of amylose will turn turbid upon standing with the formation of "retrograded amylose," which is considered a less hydrated state of amylose. Katz and Itallie (1931) found that amylopectin and amylose separated by electrophoresis from autoclaved potato starch showed retrogradation, as determined by the X-ray method. The solutions were kept for one to two months at 2°–3° C. The V spectrum of the original fractions changed to a B spectrum upon standing. They did not agree with statements in the literature that amylopectin does not retrograde. The authors did not, however, state the completeness of separation of the amyloses and a small amount of amylose in the amylopectin fraction would probably result in a change in X-ray spectrum.

As to the physical properties of the two fractions it is generally agreed that amylopectin is least soluble and forms a more viscous turbid solution which gives a red-violet color with iodine. Amylose is most soluble and least viscous and gives an almost clear solution which becomes a brilliant blue on treatment with iodine. A solution of amylose will retrograde upon standing and turn turbid, finally depositing a white precipitate. The rate of the retrogradation depends on the concentration of the solution and the temperature.

The degradations, particularly by enzymes, of the amylopectin and amylose have led to many theories as to the structure of the fractions and the degradation products. From the physical state of

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the fractions, it might be expected that the more soluble amylose fraction would be more easily hydrolyzed by enzymes. Sherman and Baker (1916) effected a separation of a 20% starch paste containing 0.002 M sodium chloride by using the centrifuge and calling the soluble fraction amylose. With fractions thus separated they found the amylose to be slightly more easily hydrolyzed by malt diastase than the amylopectin fraction. Sjoberg and Eriksson (1924) separated the fractions from potato starch by the alkali leaching method and found the amylose to be more readily hydrolyzed by amylases from both normal and germinated barley. Ling and Nanji (1925) studied the action of barley diastase on the starch fractions separated by the freezing methods and found amylose to be most readily hydrolyzed. They concluded that the difference between amylose and amylopectin is mainly steric, with the former being aggregates of alpha-hexa-amylose and the latter of alpha-beta-hexa-amylose. Josephson (1927) discussed a hypothesis that the amyloses are aggregates of elementary molecules, these units being anhydro-disaccharide for amylose, and for amylopectin a complex of the anhydro-disaccharide plus anhydroglucose.

Polak and Tychowski (1929) studied the action of amylases on amylopectin and amylose separated from potato starch by the Sherman and Baker centrifuge method. They concluded that amylopectin is hydrolyzed by alpha-amylase to give dextrin I, which has six maltose units, and that amylose is hydrolyzed by beta-amylase to maltose but by alpha-amylase to dextrin II with three maltose units. Pringsheim (1932) believed amylose is degraded to produce dihexosans and amylopectin to trihexosans, but Myrbäck and Ahlborg (1937) considered the presence of such units contrary to known facts from studies of the limit dextrins.

Samec and Waldschmidt-Leitz (1931) separated amyloamylose and erythroamylose from potato starch by electrophoresis. The amyloamylose was almost completely hydrolyzed by malt and pancreatic amylase. The erythroamylose was hydrolyzed to the extent of 70%, 96%, and 56% by pancreatic, malt, and barley amylases, respectively. Freeman and Hopkins (1936) obtained 20% amyloamylose from autoclaved potato starch by electrophoresis. This was hydrolyzed to the extent of 96.8% by barley amylase after 48 hours, while the erythroamylose fraction was only 57.7% hydrolyzed.

It appears that the previous work has been almost exclusively with amylopectin and amylose from potato starch, separated by different techniques giving fractions which probably varied considerably in completeness of separation and in properties. Hence a study of the action of the wheat amylases on wheat amylopectin and amylose was made, with separation by the electrophoretic technique.

Experimental

Many workers have failed to state the completeness of separation of the amyloses used for enzyme substrate, and in the majority of cases boiled starch solutions were used and the amylopectin fraction undoubtedly contained many entire, unruptured granules.

The fractions used as substrates in this study were prepared from commercial wheat starch which had been thoroughly pulverized by grinding in a rod mill for 84 hours. The ground starch was then fractionated by the electrophoretic method as described in Part I. The amylopectin used represented 17% of the starch and contained 0.261% phosphorus, which was 73.8% of the total phosphorus of the original starch from which it was separated. The amylose preparation represented about 80% of the total amylose obtained from the starch and contained 0.001% phosphorus. The amylases used were the purer fractions, alpha-amylase-2 and beta-amylase-2, prepared from wheat as described in Part II.

A series of hydrolysates was made, using freshly prepared substrates, and the hydrolysis was at 30° C. buffered to pH 5.1 with a citrate solution. In all instances 25 c.c. of 1% substrate and 12.5 mg. of total enzyme (same as 50 mg. per 100 c.c. of 1% substrate) or the equivalent was used. Hydrolysates with alpha-amylase alone, beta-amylase alone, and alpha- and beta-amylase combined were allowed to act for 24 hours. An equivalent amount of enzyme was then added and the hydrolysis continued up to 44 hours. The rate of hydrolysis was determined by the reducing-sugar method and expressed as percent hydrolysis in terms of the theoretically available maltose. The results are shown in Figure 1.

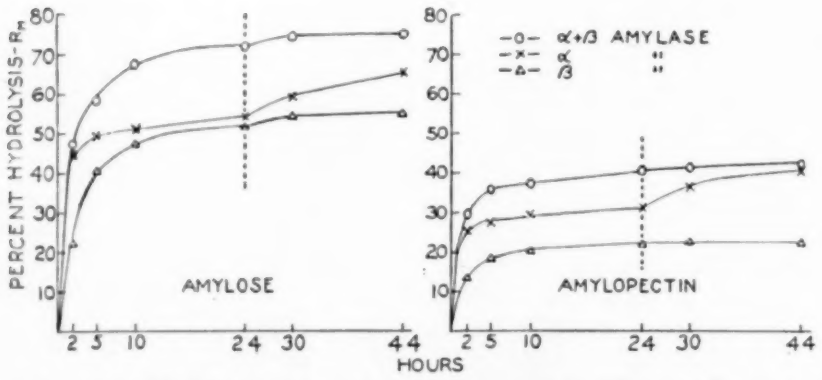

Fig. 1. Action of alpha- and beta-amylases and their mixtures on amylopectin and amylose. An additional equivalent quantity of enzyme was added after 24 hours' hydrolysis.

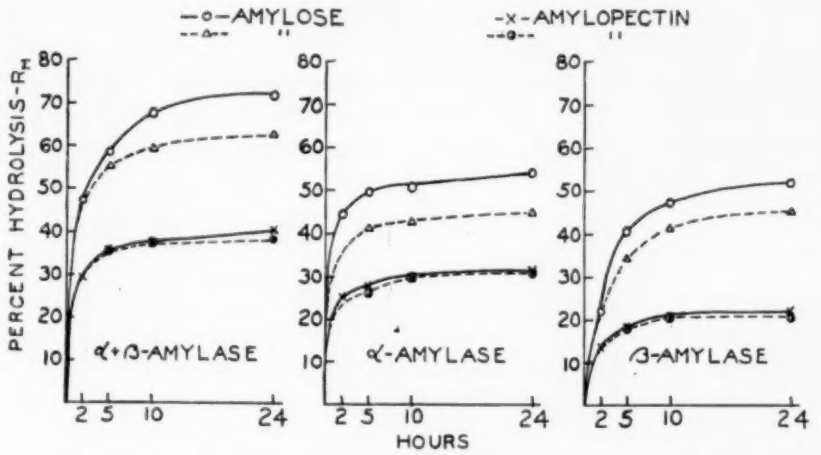

Fig. 2. The effect of retrogradation. The solid lines show the action of the amylases on freshly prepared substrates, the dotted lines are substrates which had been stored as 2% solutions for 2 days at 5°. The amylose and amylopectin were from commercial wheat starch.

It was noticed that when 2% amylose solution was allowed to stand at 5° C. it became turbid in about a day with the formation of a white precipitate. At room temperature this retrogradation required about three weeks, using a 2% solution. A study of the effect of retrogradation on enzyme susceptibility was made using both amylose and amylopectin.

TABLE I

ACTION OF BETA-AMYLASE ON AMYLOSE SOLUTIONS FRESHLY PREPARED AND AFTER STORAGE FOR 2 AND 8 DAYS AT 5° C.—HYDROLYSIS AT 30°, pH 5.1—ALL VALUES IN PERCENT HYDROLYSIS-R_M

Period of hydrolysis	Freshly prepared amylose solution	After 2 days' storage	After 8 days' storage
Hours	%	%	%
2	27.8	25.5	24.7
6	43.2	37.5	35.5
12	44.7	40.4	—
24	46.6	41.2	38.8

The rates of hydrolysis of freshly prepared solutions were determined, and 2% solutions of amylopectin and amylose, with a drop of toluol to prevent bacterial activity, were allowed to stand for two days at 5° C. and the enzyme hydrolysis was again studied. The same temperature, pH, substrate, and enzyme concentrations were used. The results are shown in Figure 2.

Amylose was precipitated by addition of alcohol to aliquots of a solution, dried, and ground to a fine powder. A few weeks later a 2% solution was made and used as enzyme substrate, freshly prepared and also after storing for 2 and 8 days respectively. Beta-amylase only was used in this instance, and the conditions were the same as for the previous tests. The results are recorded in Table I.

Discussion

The amylopectin was found to be much more resistant to the action of the amylases alone or in combination, than the amylose, and the amylopectin was more resistant to the action of beta-amylase than alpha-amylase. Combinations of alpha- and beta-amylase hydrolyzed the amylopectin slightly more rapidly than alpha-amylase alone. After 24 hours' hydrolysis, 22% of the amylopectin was hydrolyzed by beta, 31.4% by alpha, and 40.2% by alpha- and beta-amylase combined. The addition of more enzyme did not produce much further hydrolysis except with alpha-amylase, which effected 40.8% hydrolysis after 44 hours.

After 24 hours 51.8% of the amylose was hydrolyzed by beta, 54.1% by alpha, and 71.4% by alpha- and beta-amylase combined. Addition of more enzyme increased the hydrolysis by alpha to 65.6% after 44 hours, with only slight increases on like additions of enzyme to the other hydrolysates. The amylose from wheat starch was not completely hydrolyzed as was observed with amyloamylose from potato starch by Samec and Waldschmidt-Leitz (1931) and Freeman and Hopkins (1936), but the amyloamylose in the first case represented only about 50%, and in the latter case only 20% of the starch. The amylose used in this study was 83% of the wheat starch. Thus the incongruity in results is probably due to different methods of separation and different starch fractions used, as well as variations in the enzyme preparations and conditions of hydrolysis.

Retrogradation of amylose increases the resistance to the hydrolyzing action of beta- and alpha-amylases and their mixtures, as shown in Figure 2. The data in Table I indicate that most of the retrogradation took place during the first two days, and with only a slight additional change between the second and the eighth day of storage at 5° C. The storage of the 2% solution of amylopectin for two days at 5° C. produced very little change, as indicated by the rate of enzyme hydrolysis.

It is possible that wheat amylopectin and amylose differ from those separated from potato starch, in view of the theories of Samec (1934) and Koets (1935), that wheat starch contains a nitrogenous material not present in potato starch.

Summary

Amylose from wheat starch was much more easily hydrolyzed by both alpha- and beta-amylases and combinations of them than the amylopectin fraction.

Amylopectin was hydrolyzed by alpha-amylase more readily than by beta-amylase.

Amylose solutions which were allowed to retrograde for two days at 5° C. were more resistant to hydrolysis by both the amylases and their combination than was the freshly prepared amylose. Amylopectin solutions showed very little change in enzyme susceptibility after a similar storage treatment.

The decrease in enzyme susceptibility of the amylose solution was most rapid within the first two days of retrogradation at 5° C.

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THE USE OF DIELECTRIC MEASUREMENTS TO DETERMINE THE MOISTURE CONTENT OF POWDERY SUBSTANCES

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Very many food products such as flour, granulated sugar, starch, meal, grain, and others are powdery or friable substances. Besides, many products like biscuits, lump sugar, rusks and others can easily be converted into powders. Moisture determinations on such products are frequently made in food laboratories, and this circumstance has led us to choose these products as materials for research work in the determination of moisture content by the dielectric constant method.

Many investigators have studied the problem of determining the dielectric constant (DK) of powdery substances. Thwing (1894) was the first to determine the DK of powdery substances in a mixture with alcohol or ether. He made calculations according to the rule of mixtures. Starke (1897) employed isoelectric liquids and obtained good results for substances which are non-conductors of electricity. Loewe (1898) should be mentioned among other investigators and in particular J. Tausz and Rumm (1934), who carried on extensive work in the study of the DK of certain powdery and fibrous substances. These investigators, however, did not assume the task of using DK measurements for determining moisture content. While some of them pointed out such a possibility, no investigations on this problem were made.

Berliner and Rüter (1929) were the first to use DK measurements for a practical purpose, and they recommended that the moisture content of flour and grain be determined by the DK method. They obtained results which indicated that DK measurements were wholly applicable for the determination of moisture content with sufficient accuracy for practical purposes.

Other investigators confirmed their findings. This, during the past five years, has led to the appearance of many dielectric-measurement instruments for the determination of moisture content. While these instruments made for very rapid and technically accurate determinations of moisture content, they had the shortcoming of being too specialized, that is, of being suitable only for the one substance for which their scales were calibrated. No general methods for determining the DK and therefore the moisture content of any powder have as yet been elaborated.

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The principle underlying the determination of a DK which is due in part to moisture is not different from that underlying the determination of the DK of ordinary dielectrics. The dielectric constant of dry substances classified as food products attains a magnitude of not more than 8 to 10, while the DK of water approximates 80. It follows from this that a change in the composition of dry substances affects the DK of the entire substance very little, while a slight increase or diminution of the moisture content immediately raises or lowers its DK. This statement does not hold for substances containing colloids and consequently able to combine with water, with a resultant large reduction of the DK of the water in combination. In cases therefore in which the moisture content is slight, *i.e.*, when the water is mostly held in combination, the DK change with variable moisture content is very slight and quite out of proportion to the DK of water. This greatly complicates the use of the dielectric method. This complication is also met of course in dealing with our powdery substances, but there are other specific difficulties which must be overcome if the purpose of obtaining satisfactory estimation of moisture is to be attained.

The following method of determining the moisture content of powders by DK can easily be imagined schematically. A test powder is poured into a condenser having a certain electrical capacity. This condenser is connected to an instrument, and the increase of its capacitance is determined. The moisture content sought is calculated according to a previously drawn curve showing the degree to which the DK of the powder or the capacitance of the condenser is dependent on the moisture content of the powder.

The entire measurement would require literally one minute were it not for the following complications. First, the DK of substances which contain water depends greatly on the temperature and hence the temperature must necessarily either be held constant or else corrections must be made for its changes. Second, the powder is not a homogeneous substance, like a liquid, but is a mixture of the powder substance itself and air. The dielectric constant of such a two-component dielectric will depend on the ratio of the component parts and consequently in any given case on the magnitude of compression and the size of the powder particles.

The first difficulty can easily be overcome. The second, however, is one of the chief obstacles standing in the way of determining with satisfactory accuracy the moisture content of powders. Before beginning to work out a method suitable for determining the moisture content of any powder, it is first necessary to devise a method of eliminating the effect which variations in compression have on the results.

The simplest solution, it seems, would be to have identical compression when the powder is poured into the condenser. But this is extremely difficult to attain. There are two ways: either to pour at all times without compression or to apply a definite degree of compression which has at all times the same magnitude (Henriquez and Renaud, 1935). In both cases special devices are required and this complicates the entire instrument. Moreover, the use of such devices is not feasible, because difficulties are encountered in that the degree of crumbling (*i.e.*, the size of the particles) affects the density and the ratio of the volumes which the substance and the air occupy. Hence identical compression cannot be obtained.

Wholly satisfactory results can be obtained only when crumbling is identical, a condition which is very seldom met with in practice; however, in most cases the degree of compression can be determined mathematically.

The problem can be approached from two angles. The powder can be regarded as a mixture of two elements—the powder substance itself and air. With the DK of such a mixture and the ratio of the volumes of the components, the dielectric constant of the powder substance can be calculated by a special formula. Or one can assume the powder to be a homogeneous dielectric and make no calculation of the DK of the substance, corrections for the degree of compression being made directly in the DK of the powder. There are many formulas by which the DK of the substance may be calculated from the DK of the mixture. They were worked out on the basis of the rule of mixtures. Examples are those of Lorenz-Lorentz, Beer, Newton, Silberstein, and Lichtenecker. See Lichtenecker (1926) and Bruggeman (1936).

Only the Lichtenecker formula has proved theoretically suitable for our purpose, since the use of the others is limited by many conditions (such as the form of the particles, the correlation of the parts, and so on). While Tausz and Rumm pointed out in their work (1934) that the formula of Lichtenecker gives good results for powders with particles of arbitrary form, they did not confirm this conclusion with sufficient experimental material. The following equation expresses the logarithmic rule of mixtures on which the formula of Lichtenecker is based.

$$E_m = E_1^{\delta_1} \cdot E_2^{\delta_2}$$

where E is the DK of the mixture and E_1 and E_2 are the DK values of the component parts, and δ_1 and δ_2 are fractions of the volume occupied by the parts. If one substance is air, having a DK almost equal to unity, and if E_2 is taken as the DK of air, then

$$E_m = E_1^{\delta_1}$$

and $$\log E_m = \delta_1 \log E_1$$

or $$\log E_1 = \frac{\log E_m}{\delta_1} \qquad (1)$$

Consequently, knowing the DK of the mixture and δ_1, the fraction of the total volume which the substance itself occupies, one can use this formula to calculate the DK of a substance. The value of δ_1 can be determined if one knows the weight of the substance in a certain volume and its specific gravity, since

$$\delta = \frac{a}{vd}$$

where a is the weight of the substance, v is the volume occupied by the mixture (substance and air), and d is the specific gravity of the substance.

The same logarithmic rule can be used to make corrections for compression directly in the DK of the mixture. For this purpose we made the following transformation in the formula of Lichtenecker. In place of

$$\log E_m = \delta_1 \log E_1$$

one can write

$$\log E_m = \frac{a}{vd} \log E_1$$

where E_m is the dielectric constant of the mixture of the powder and air, a is the weight of the powder poured in the condenser, v is the volume of the condenser, and d is the specific gravity of the substance.

For the same powder, but where the degree of compression differs and consequently the weight also, the formula is

$$\log E_m^1 = \frac{a_1}{vd} \log E_1$$

where E_m^1 is the new dielectric constant of the mixture of powder and air and a_1 is the new weight of the powder poured into the condenser. On dividing one equation by the other one obtains:

$$\frac{\log E_m}{\log E_m^1} = \frac{a}{a_1}$$

and after solving for $\log E_m$ one obtains

$$\log E_m = \frac{a \log E_m^1}{a_1} \qquad (2)$$

If one knows the DK of a powder for one degree of compression (E_m^1), this formula can be used to calculate the dielectric constant of the

powder as such (E_m) for any degree of compression. The weight of the powder contained in the condenser characterizes the degree of compression.

Hence if any weight, 100 grams for instance, be taken as a comparative base, the results of the various tests can be recalculated and expressed as comparative results which would have been obtained had 100 grams of the material filled the condenser. Thus the influence of compression can be taken into account and comparative data obtained. Then the formula (2) takes the following form:

$$\log E_m = \frac{100 \log E_m^1}{a_1}$$

This formula has the advantage over the first (1) that it does not require that either the volume occupied by the substance or therefore the specific gravity of the substance be determined. Simplification of this nature results at the expense of generality since now only relative values reduced to the chosen weight of 100 grams are obtained. However, this formula yields results which differ only slightly from those obtained with the generalized Lichtenecker formula. This of course should be expected since the same logarithmic rule of mixture is fundamental to both formulas.

Since these results did not always prove satisfactory enough, we used the following formula, which turned out to be more suitable for the products investigated by us.

$$\log E_m = \frac{a^1 \log E_m^1}{a_1^1}$$

or when a equals 100 grams,

$$\log E_m = \frac{100^1 \log E_m^1}{a^1} = \frac{21.54 \log E_m^1}{a^1}$$

By using formulas to recalculate the findings in our work, we assumed the task of verifying experimentally the possibility of calculating the effect of compression on the DK of powders, and hence the possibility of determining their moisture contents accurately. Biscuits, flour, and granulated sugar served as research materials. The "Dielkometer," made by the Haardt firm, was used to measure the DK. The accuracy of the capacitance readings attained on this instrument is very great, reaching 0.001 cm. and less.[2] So from this viewpoint the instrument was very suitable for our purpose. Its shortcoming, however, is that it gives really precise measurement of DK only for

[2] A capacitance of 1 cm. = 10/9 micro microfarads.

dielectrics with slight electrical losses, that is, for non-conductors of electricity, such as many organic liquids—benzol, toluol, alcohol, or solid substances which only slightly absorb the electric vibrations.

But for substances with considerable losses the instrument gives more or less approximate values. This is due to the effect which the electrical loss has on the frequency of vibrations of the generator, which is the basic part of the instrument. On the Dielkometer, for example, we obtained the following data on the effect which the electrical conductivity of water and alcohol has on the resultant measurements of DK. The electric conductivity was varied by adding a one percent solution of potassium chloride in varying quantities. In the case of alcohol, the effect of the water added to the solution was also taken into account. Consequently the dependence on conductivity is very considerable.

Liquid Used	*DK*
Distilled water	80.00
100 c.c. H_2O plus 0.04 c.c. 1% KCl	80.91
100 c.c. H_2O plus 0.08 c.c. 1% KCl	82.24
100 c.c. H_2O plus 0.12 c.c. 1% KCl	82.80
100 c.c. H_2O plus 0.20 c.c. 1% KCl	81.54
100 c.c. H_2O plus 0.24 c.c. 1% KCl	80.98

Liquid Used	*DK*
94 per cent alcohol	30.72
10 c.c. alcohol plus 0.04 c.c. 1% KCl	30.58
10 c.c. alcohol plus 0.28 c.c. 1% KCl	30.44
10 c.c. alcohol plus 1.2 c.c. 1% KCl	30.72
10 c.c. alcohol plus 1.8 c.c. 1% KCl	31.00
10 c.c. alcohol plus 4.0 c.c. 1% KCl	31.70

Graffunder and Weber (1931) present data indicating that the frequency of vibrations and therefore also the magnitude of the apparent DK of the substance depends on electrical conductivity. These authors propose a method of their own to eliminate this influence by attaching a device to compensate for the loss. The Dielkometer has no such device. Since the loss was fairly large in the products tested by us, rising with an increase in moisture content, we have to face the fact that what we have measured is not the true capacity but rather an approximate value.

The test powder was poured in a measuring condenser, made of two plates which were placed in a vessel having a mark near the top (Fig. 1). The vessel was fixed in an ebonite framework, making it possible to connect the condenser to the Dielkometer at all times in the same way. This is very important for precision measurements. The plates were 95 by 36 by 1.5 mm. in size and were placed 10 mm. apart. Up to the mark the vessel had a volume of 237 c.c. The empty condenser weighed 313.2 grams.

The Dielkometer is provided with two comparison condensers: a "grobkondensator" for coarse measurements, and a "feinkondensator" for precise measurements. In our work the correlation between the two comparison condensers was found, and the experimental condenser (Fig. 1) was then calibrated in terms of the "grob-

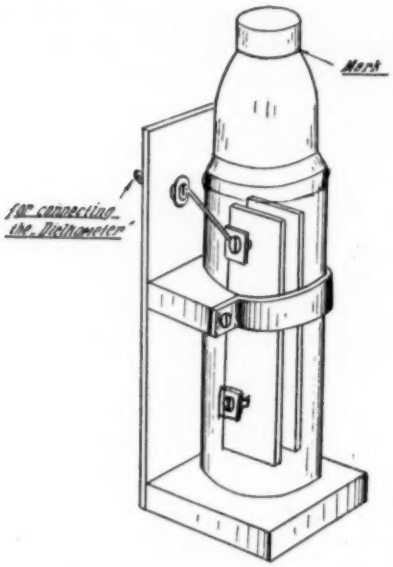

Fig. 1. Measuring condenser.

kondensator" alone, using mixtures of benzol and alcohol of known dielectric constants. The calibration curve is shown in Figure 2.

Instead of using the calibration curve as such, we found it more accurate to use its equation:

$$DK = 12.915 - 0.2186\,n$$

where n is the reading of the comparative condensers (reduced to the "grobkondensator").

In order to avoid large fluctuations of temperature during the measurements the whole instrument was placed in a wooden case which was heated inside by electric lights. However, having in view the practical task of determining the moisture content by the DK and limiting ourselves by control measurements of the capacity of the empty condenser before the test, we did not aim to attain absolute constancy of temperature. The deviations from the indices, which were calculated when the calibrated curve was drawn up, gave corrections for temperature variations and for fluctuations in the voltage

of the battery. As the experiment showed, this was quite enough for practical work at a moderately fluctuating temperature (± 2°–3°).

The powder was poured in up to the mark and slightly compressed (after which additional powder was poured in to fill up to the mark). The condenser was then connected to the instrument and a reading was taken, after which the full condenser was weighed on a technical balance having an accuracy of 0.1 gram. By deducting the weight of the empty condenser we obtained the weight of the powder.

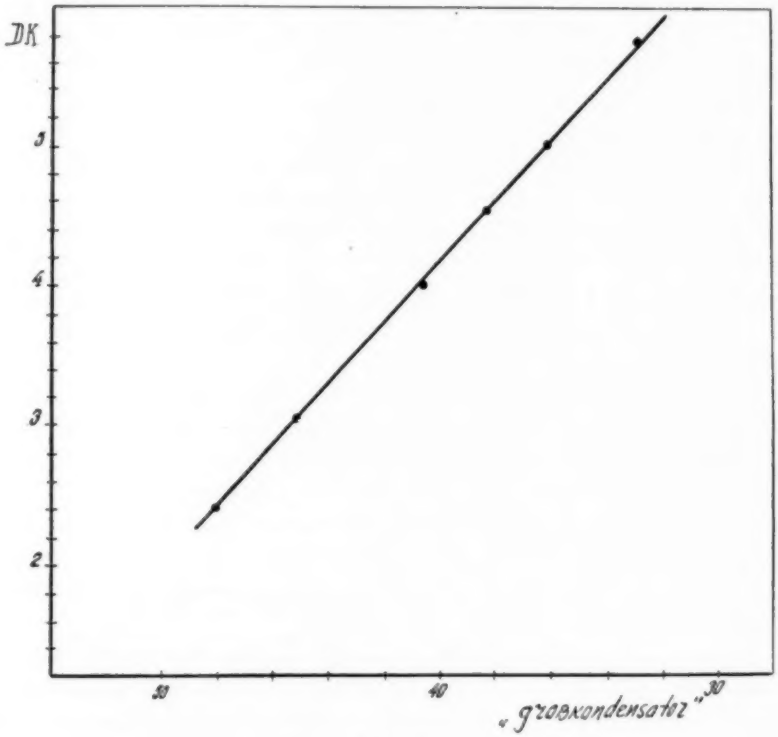

Fig. 2. Calibration curve.

By picnometric means in toluol we determined the specific gravity of the substance, which was essential in order to calculate the DK of the substance by the logarithmic formula. For each variety of material tested one common specific gravity was taken for samples of varied moisture content. The specific gravity, it should be noted, was somewhat reduced with an increase in moisture, but not enough to interfere with taking on average specific gravity for the entire series of tests.

The following powdery substances served as test materials:[3]

Ground biscuits, the sugary sort No. 1.
Ground biscuits, the sugary sort No. 2.
Ground biscuits, the sugary sort No. 2 (samples ground coarser than in previous case).
Ground biscuits, hard sort No. 3.
Wheat flour, 85% roughage.
Wheat flour, 79% roughage.
Granulated sugar.

A curve was first drawn for each of these substances showing the degree to which the dielectric properties depended on the moisture content. For this purpose we did as follows. The moisture content of the initial sample of each product was determined by the method employed in laboratories of the confectionary industry. Biscuits and flour were dried for 40 minutes at a temperature of 130 degrees. Granulated sugar was dried to a constant weight at a temperature of 100 to 105 degrees. The average test sample was poured in the measuring condenser up to the mark. The condenser was weighed and connected to the Dielkometer, and a reading of the capacity was taken on the comparative condenser. The pouring and readings were repeated five times.

After this, the initial sample was divided into two parts, one of which was placed in a water-containing desiccator for a certain length of time, resulting in a gradual increase in the moisture content of the substance. The other part was dried at a low temperature. After some time had elapsed and the substances had been stirred with great care, two samples of the same original product were obtained: one part having a moisture content greater and the other less than the initial sample.

These were tested in exactly the same way as the original samples. They were poured into the condenser, weighed, and a reading of capacity was taken. After this the samples with increased moisture contents were again made more moist, and those with lower moisture contents were dried further. Thus, two samples were again obtained, one having a still lower and the other a still greater moisture content. The results of measurements on the Dielkometer were recalculated by formula and an average was obtained for each sample. Thus the points of the curves were obtained.

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[3] Recipes of dough of sugary sorts: 320 kg. of 30% wheat flour, 110 kg. powdered sugar, 0.3 kg. ammonium carbonate, 55 kg. butter, 2.2 kg. soda, 2.0 kg. salt, 2.0 kg. vanilla powdered sugar, 24 kg. eggs, 16 kg. starch, 40 kg. milk, 16 kg. lactins, 15 kg. invert sugar.

Recipes of dough of hard sorts: 320 kg. flour, 72 kg. powdered sugar, 32 kg. starch, 32 kg. butter, 2.8 kg. salt, 2.8 kg. soda, 0.6 kg. ammonium carbonate. 0.5 kg. vanilla powder, 12 kg. eggs, 90 liters milk, 6 kg. treacle.

To present all the calculations and results of the measurements in a given test would be too cumbersome. They number several hundred. Hence, we present in full but three examples, from which the sequence and essence of the tests are evident. Of the remaining experiments, however, only average figures will be presented. Table I shows the results of measurements for the dampened sample of Biscuit No. 1 with a moisture content of 10.76%. The same is given in Table II for the dried flour and in Table III for the granulated sugar.

TABLE I

BISCUIT NO. 1, MOISTURE CONTENT 10.76 PERCENT

Readings on "Grobkondensator" 1	Readings on "Feinkondensator" 2	Summary readings in terms of "Grobkondensator" alone 3	DK of mixture of substance and air [1] 4	Weight of condenser with substance 5
46.05	50.0	46.05	2.848	432.5
46.05	51.5	46.10	2.838	432.9
46.05	53.9	46.19	2.818	431.3
46.05	57.8	46.32	2.790	429.3
46.05	48.0	45.97	2.866	432.3

Weight of substance 6	Specific gravity of substance 7	$\delta = a/vd$ 8	DK of substance calculated by Lichtenecker formula 9	DK of mixture reduced to 100 g. according to our formula 10
119.3	1.346	0.374	16.45	2.523
119.7	1.346	0.375	16.14	2.522
118.1	1.346	0.370	16.45	2.526
116.7	1.346	0.366	16.50	2.522
119.1	1.346	0.373	16.82	2.522

[1] Calculated from the equation of the calibration curve (Fig. 2).

TABLE II

FLOUR, MOISTURE CONTENT 9.0 PERCENT

Readings on "Grobkondensator" 1	Readings on "Feinkondensator" 2	Summary readings in terms of "Grobkondensator" alone 3	DK of mixture of substance and air [1] 4	Weight of condenser with substance 5
46.8	50	46.8	2.685	448.4
46.8	48.4	46.74	2.698	447.9
46.8	52.8	46.90	2.663	445.9
46.8	52.3	46.88	2.667	446.6
46.8	51.9	46.87	2.669	444.9

[1] Calculated from the equation of the calibration curve (Fig. 2).

TABLE II—*Continued*

Weight of substance 6	Specific gravity of substance 7	$\delta = a/vd$ 8	DK of substance calculated by Lichtenecker formula 9	DK of mixture reduced to 100 g. according to our formula 10
135.2	1.439	0.396	12.11	2.270
134.7	1.439	0.395	12.34	2.256
132.7	1.439	0.389	12.40	2.250
133.4	1.439	0.391	12.30	2.250
131.7	1.439	0.386	12.74	2.263

TABLE III

GRANULATED SUGAR, MOISTURE CONTENT 0.55 PERCENT

Readings on "Grobkondensator" 1	Readings on "Feinkondensator" 2	Summary readings in terms of "Grobkondensator" alone 3	DK of mixture of substance and air [1] 4	Weight of condenser with substance 5
49.3	50	49.3	2.138	494.8
49.3	47.0	49.18	2.164	498.4
49.3	47.1	49.18	2.164	497.4
49.3	42.8	49.01	2.201	502.1
49.3	44.8	49.09	2.184	500.8

Weight of substance 6	Specific gravity of substance 7	$\delta = a/vd$ 8	DK of substance calculated by Lichtenecker formula 9	DK of mixture reduced to 100 g. according to our formula 10
181.6	0.483	1.588	4.60	1.640
185.2	0.493	1.588	4.79	1.668
184.2	0.490	1.588	4.83	1.671
188.9	0.502	1.588	4.81	1.675
187.6	0.498	1.588	4.82	1.671

[1] Calculated from the equation of the calibration curve (Fig. 2).

Such measurements and calculations were made for every sample tested. Table IV presents the results of the measurements for all the products tested. Each figure is the average of five experiments.

The curves corresponding to these tables are given in Figures 3, 4, and 5. The curves of Figure 3 show the degree to which the DK of the mixture (powder) depends on the moisture content, disregarding compression. The figures under column 2 of Table IV were used to draw the curves. The curves of Figure 4 show graphically the degree to which the DK of the substance calculated by the logarithmic formula depends on moisture content (data under column 3 used). The curves of Figure 5 show the degree to which the DK of the mixture,

TABLE IV

MEASUREMENTS FOR ALL PRODUCTS TESTED

Moisture percentage (by drying) 1	DK of mixture of the substance and air 2	DK of substance calculated by logarithmic formula 3	DK of mixture reduced to 100 grams according to our formula 4
	Biscuit No. 1		
1.89	1.973	5.35	1.775
2.37	1.990	5.38	1.779
9.25	2.561	13.15	2.339
10.76	2.823	16.47	2.529
13.46	3.404	27.27	3.044
15.75	3.719	35.40	3.345
	Biscuit No. 2 (finely crumbled)		
3.89	2.198	5.64	1.846
4.66	2.291	6.37	1.921
9.00	2.605	10.45	2.240
10.71	2.937	13.52	2.412
11.59	3.226	15.96	2.592
13.93	3.567	25.60	2.989
	Biscuit No. 2 (more roughly crumbled)		
3.18	2.009	6.12	1.839
6.64	2.247	7.63	2.004
9.37	2.562	13.28	2.349
11.61	2.820	18.63	2.603
12.41	3.201	24.34	2.832
13.91	3.438	33.45	3.147
	Biscuit No. 3		
3.14	1.918	6.05	1.834
4.79	2.060	6.72	1.919
8.75	2.405	11.28	2.260
14.37	3.512	33.72	3.252
16.77	4.154	64.50	3.950
	Flour, 85%		
3.59	2.251	8.40	1.986
4.45	2.300	8.52	1.994
9.60	2.855	13.87	2.350
12.75	3.029	18.74	2.549
13.67	3.050	20.76	2.611
14.67	3.251	23.84	2.735
15.00	3.350	26.38	2.798
	Flour, 79%		
3.44	2.133	8.28	1.924
3.84	2.238	8.58	1.981
9.00	2.676	12.38	2.258
12.77	2.921	18.88	2.529
13.84	3.039	21.48	2.624
15.55	3.283	28.18	2.839
16.10	3.322	30.15	2.891

TABLE IV—*Continued*

Moisture percentage (by drying) 1	DK of mixture of the substance and air 2	DK of substance calculated by logarithmic formula 3	DK of mixture reduced to 100 grams according to our formula 4
	Flour, 30%		
3.58	2.369	8.78	2.031
5.57	2.502	10.28	2.137
8.10	2.849	12.49	2.299
15.71	3.285	24.64	2.768
17.62	3.370	30.22	2.930
	Granulated sugar		
0.05	2.015	3.71	1.553
0.55	2.170	4.77	1.665
1.00	2.307	5.78	1.762

reduced to 100 g. according to our formula (column 4), depends on the moisture content.

Having obtained the experimental results, the next problem was to compare the accuracy with which the moisture content of the powders could be determined by the three methods.

It was essential first of all to know how accurate the determination of moisture content was when no estimate was made of the compression. Since this method is the simplest and most rapid, it would be the most practical, were it to yield good results. If the accuracy of this method be compared with the accuracy of analysis obtained when the formulas were used, appropriate conclusions can be drawn as to the expedience of the recalculation by the formulas. Besides it is essential also to throw light on the problem of the effect which crumbling has in each case.

To determine the accuracy of analysis in cases where the crumbling was identical, we set out in the following way. Each point of the curve showing the degree to which the DK depends on the moisture content represents the average of a number of condenser readings at the indicated moisture content. The difference between the average and the moisture content corresponding to each reading gives the degree to which the result of the given measurement deviates from the average. The maximum difference in the results is the difference between the greatest and the least values. If the arithmetic averages of all the deviations obtained are taken and classified according to kinds of product, then the following data are obtained (Table V).

From these figures it is obvious that when the degree of compression is taken into consideration, the results are considerably more satisfactory. For ordinary pouring the average maximum deviation reaches 0.43% for biscuits, 0.95% for flour, and 0.18% for sugar. But should the data of individual tests be taken instead of average values, these figures are obtained: 1.7% for flour up to 0.9% for biscuits, and up to 0.19% for granulated sugar. This is evidence that

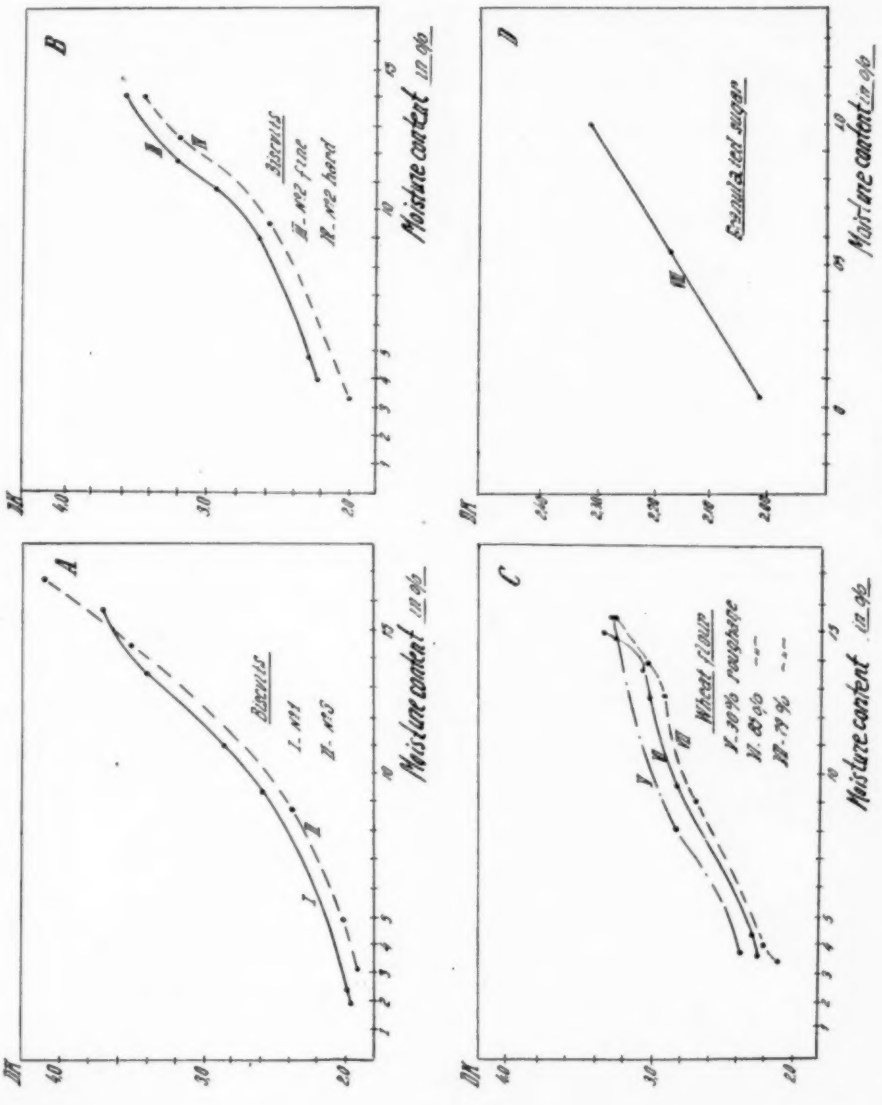

Fig. 3. Relationship of DK to moisture content.

only approximate data can be obtained with such a method. When a recalculation of individual tests is made by the logarithmic formula, the divergence becomes less, reaching 0.40% for biscuits, 0.70% for flour, and 0.06% for sugar. When our formula is applied it becomes still less, reaching 0.20% for biscuits, 0.55% for flour and 0.10% for sugar. Consideration of the degree of compression in the case of crumbled biscuits proves especially effective.

The following conclusion can be drawn from the facts obtained. In cases in which the crumbling is identical the moisture content of

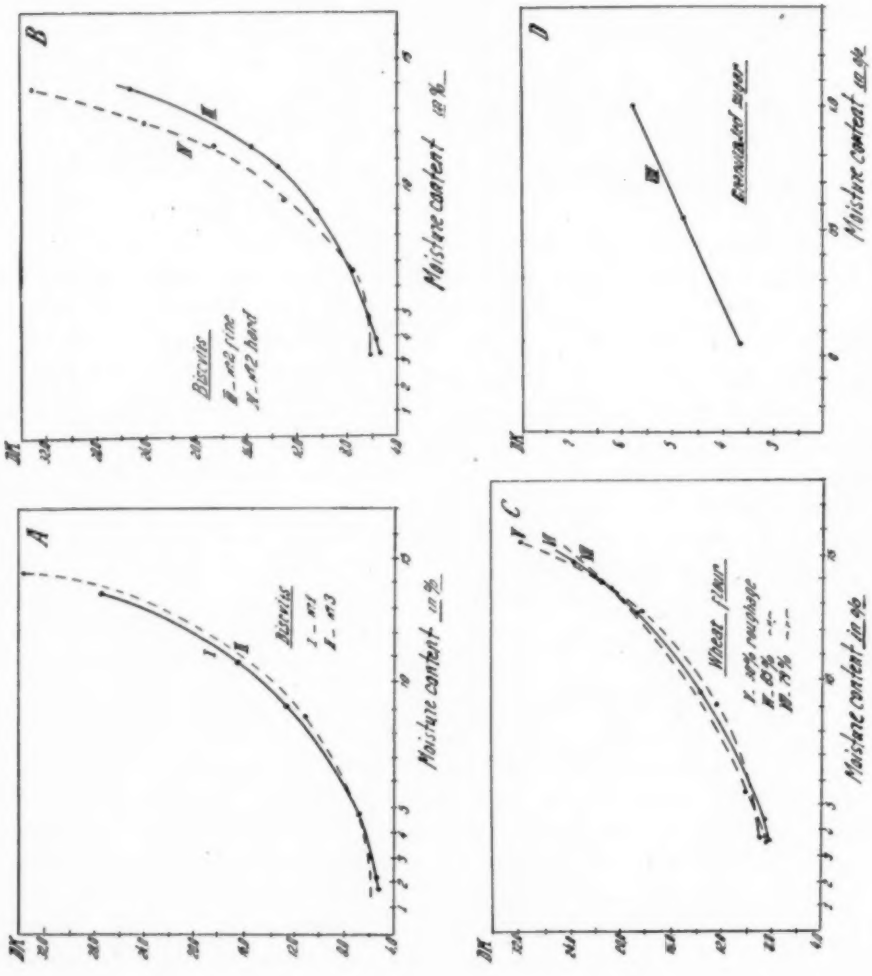

Fig. 4. Relationship of DK calculated by logarithmic formula to moisture content.

biscuits, flour, and granulated sugar can be determined satisfactorily by DK method when calculations are made, either by the largarithmic formula or by our formula. The average deviation from the real (0.09 and 0.07 for biscuits, 0.19 and 0.16 for flour and 0.04 for sugar) is altogether practical for industrial purposes.

A somewhat different picture is obtained when the degree of crumbling varies. From the curves drawn for the two sorts of Biscuit No. 2, which vary in degree of crumbling, one can judge the influence of the size of the powder particles when compression is disregarded (see curve B, Fig. 3). A systematic difference is obtained also when the logarithmic formula is used (see curve B, Fig. 4). Consequently

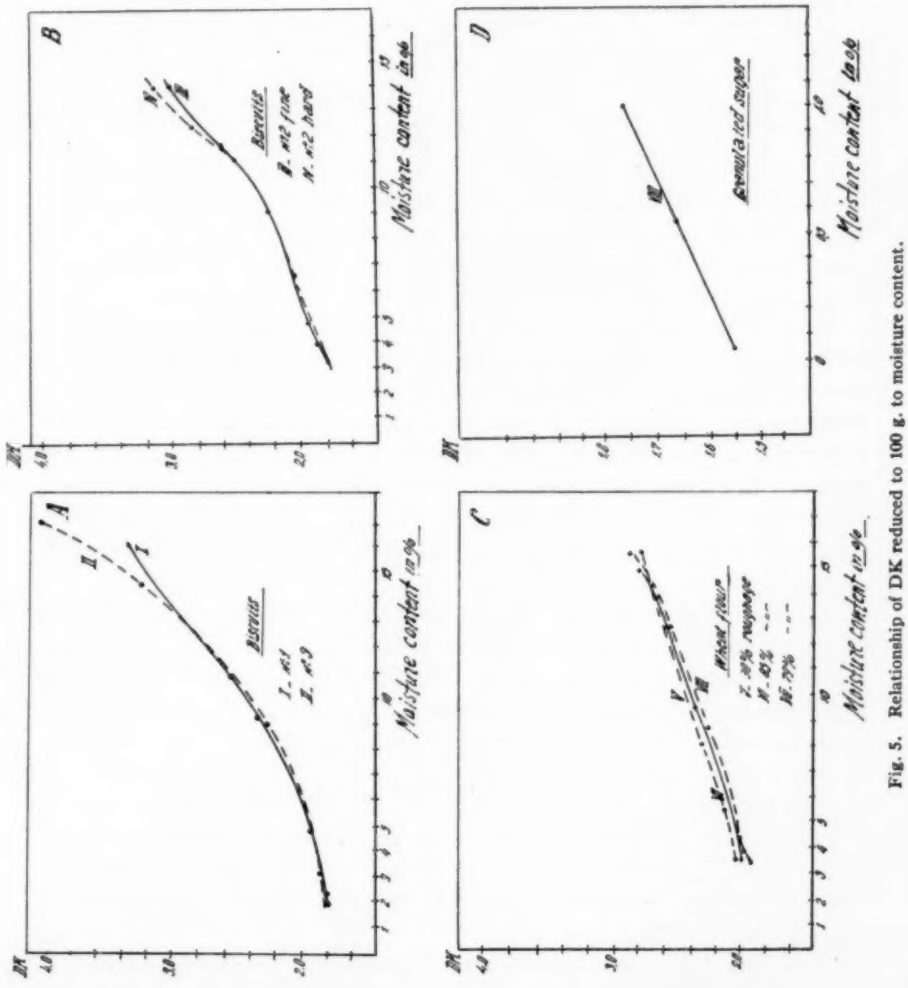

Fig. 5. Relationship of DK reduced to 100 g. to moisture content.

Fig. 4. Relationship of DK calculated by logarithmic formula to moisture content.

TABLE V

AVERAGE MAXIMUM DEVIATION IN PERCENTAGE OF MOISTURE CONTENT

Substance	Without calculation of compression	With calculation by logarithmic formula	With calculation by our formula
1	2	3	4
Biscuits	0.43	0.28	0.19
Flour	0.95	0.54	0.48
Granulated sugar	0.18	0.06	0.10

AVERAGE, TAKEN FOR ALL MOISTURE VALUES, OF THE AVERAGE DEVIATION OF INDIVIDUAL MOISTURE VALUES IN PERCENTAGE OF MOISTURE CONTENT (MEAN AVERAGE DEVIATION)

1	2	3	4
Biscuits	0.20	0.09	0.065
Flour	0.31	0.19	0.16
Granulated sugar	0.05	0.04	0.035

it is apparent that this formula makes no compensation for variations in the degree of crumbling. With moisture contents in the range most probable in practice wholly satisfactory results were obtained with this biscuit only when our formula was applied. This is apparent from the satisfactory coincidence of the curves B of Figure 5.

The problem of determining the moisture content of powders by DK regardless of compression and size of particles must not of course be regarded as fully solved. Considerable work must still be done in testing the applicability of the formulas, in studying the degree to which the results obtained depend on different conditions and factors, on the character and composition of the powder, etc. However, judging from the wholly satisfactory results we obtained, in particular for biscuits, there are grounds to hope for complete success in future work.

It should be noted that apart from our direct purpose, the experimental material can be used also for other conclusions. For instance, the slow rise and subsequent acceleration of DK curves in case of slight moisture content is evidence of the effect of colloids, which combine with water and in this way reduce its DK.

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REPORT OF THE MALT ANALYSIS STANDARDIZATION COMMITTEE

E. SINGRUEN

Modern Brewer, New York, N. Y.

(Read at the Annual Meeting, May 1938)

The event placing me today into the position of Acting Chairman of the Malt Analysis Standardization Committee of the A. A. C. C. is one that fills us with grief. I refer to the loss of a true friend and always cooperative associate, occasioned by the unexpected and untimely death of Dr. D. A. Coleman. Appointed in 1934 as chairman of the then newly formed Committee on Standardization of Methods for Brewing and Malting Control, Dr. Coleman organized and successfully conducted this important work until his passing last February.

It was my good fortune to have been closely associated with him in this work. Only a few weeks prior to his taking ill, I had the opportunity of visiting him in Washington, at which time he outlined this program and discussed with me the collaborative analytical work to be conducted for the annual report of the committee, which we are about to hear.

In accordance with his plans, five samples of malt with diastatic powers ranging from 40° to 170°L. were sent out to 22 laboratories with the request to determine the diastatic power by the method used for routine tests. The remainder of the samples was to be kept to permit further study of the same malts based on the suggestions offered here today.

Because of the limited time at our disposal in which to conclude this study, it was decided to confine our tests to the determination of the

diastatic power, which had in previous years shown the greatest variations in results.

At this time I take the opportunity of thanking all collaborators who participated in this work for their generous cooperation and for the prompt response necessitated by the rapidly approaching date of this meeting. Of the twenty-two laboratories nineteen sent in their results: among them were seven malt houses, three breweries, three scientific stations, and four government agencies.

After careful study, the results were tabulated, multigraphed, and returned to all collaborators for comment. Thirteen replies were received. Therefore, this report reflects not only the opinion of the committee but includes the comments and suggestions of the majority of the collaborators. The fact is that despite the great strides toward standardization of procedures for the determination of diastatic power in malt, 7 different methods and 4 modifications are in actual use as routine procedures in the various laboratories. This emphasizes the distinct need for agreement on one standard or reference method with which to compare other alternate procedures. The chief variation exists in the determination of the maltose formed.

PARTIAL SUMMARY OF RESULTS FOR DETERMINATIONS OF DIASTATIC POWER

A.S.B.C. Method for Diastatic Power

Lab. No.	5	6	7	8	17	Max. dif.	Mean
Malt 1	59	45	43	53	51	16	50
2	81	68	69	65	74	16	79
3	111	94	113	110	111	19	108
4	130	129	154	146	136	25	139
5	143	161	179	162	152	36	159

Sallans and Anderson, Ferricyanide Method for Diastatic Power

Lab. No.	3	15	16	Max. dif.	Mean
Malt 1	47	46	46	11	47
2	71	74	73	3	73
3	109	113	112	4	111
4	141	137	137	4	138
5	159	164	158	6	160

By the accumulation of collaborative results obtained by different methods, valuable data have been gathered which permit a better evaluation of the individual procedures. Preference was given to the ferricyanide method as suggested by Sallans and Anderson (Can. J. Research **C 15**: 70–77, 1937, and Modern Brewer, Nov. 1937) and to the method of the A.S.B.C. In addition gravimetric, tube, and polarimetric methods were employed by individual laboratories on the basis of personal preference or adaptability to existing conditions. If these individual methods are eliminated the mean results of the ferricyanide and A.S.B.C. methods are in close accordance, with less variation in results with the ferricyanide method, both for repeat tests

in one laboratory as well as for comparative tests by different analysts.

Based on the results and comments of the collaborators the committee proposes the following recommendations:

1. That the study of procedures for the determination of diastatic power in malt be continued on the same samples now in the hands of the collaborators before action is taken on adopting one or the other method as standard.
2. That the ferricyanide method as published by Sallans and Anderson be compared with the method of the A.S.B.C. without modifications in either case.
3. That groups be formed according to the preference of different laboratories for a specific method to study the procedures; this would include the polarimetric, gravimetric, and ceric sulfate methods.
4. That all methods, together with their modifications, be made available in either multigraphed or printed form to give all collaborators the benefit of closer study.

CEREALS USED IN BREWING

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(Read at the Annual Meeting, May 1938)

When we speak of cereals in connection with brewing, we usually think of barley, malt, corn, and rice and give little thought to the fact that practically every known cereal, with the exception of wild rice, at some time or other has been used in the manufacture of fermented beverages comparable with beer.

Cereal beverages have formed an integral part in the diet of the human race throughout the ages. They were the tribal or national beverage of all agricultural peoples and were considered as essential as bread. While bread filled the need for sustaining and energy-providing food, these fermented beverages contributed to the enjoyment of life in the leisure hours. In many cases they contributed greatly to the maintenance of health by taking the place of unsatisfactory or infected water supplies.

They also played an important part in the worship of primitive people and in the celebration of important events and festive occasions such as weddings and births, as well as a religious and national feasts and holidays. The almost universal use of barley malt can probably be attributed to the fact that the culture of barley is possible further

north and at higher altitudes than any other grain—as well as to the comparative ease with which it can be malted.

Ancient Cereal Beverages

Records of beer brewed with barley date back to 2017 B.C. when the tribes along the Nile at Pelusium made a beverage from it, sweetened with honey. In 1960 B.C., Osiris introduced "zythos," a fermented beverage made of barley.

During the time of the pharaohs brewing was an important industry in Egypt and many distinctly different brewing formulas have come to us in hieroglyphics, relating in detail materials and brewing processes used. These documentary scripts lead us to believe that red barley was the chief constituent of the "hek," "hag," or "bausa" of the old Egyptians. G. Maspers, an Egyptologist, reports that "Beer has always been the favorite beverage of the people. Sweet beer, iron beer, sparkling beer, perfumed and spiced beers were drunk cold or hot, beer of thick, sticky millet like that prepared in Nubia and among the negroes of the Upper Nile."

The grain was crushed in a mortar and moistened; then lumps of this dough were thoroughly kneaded with the feet in a big pot. The wort resulting from an infusion of this dough was poured through a sieve, consisting of a wide, flat basket resting on a large container. Finally it was poured into high jugs which were sealed with large cones of Nile mud.

"Banzali," the beer of the natives in modern Egypt, is produced by essentially the same methods as were practiced 3,000 years ago. Barley, wheat, or millet is used in malted or unmalted condition. Color and flavor are imparted by pungent spices. It is unknown whether in old times the spices were added to the beverage as an ingredient or were eaten with the drink.

All Oriental and African races had beverages made from cereals or cereal products, frequently sweetened with honey and flavored with aromatic vegetable substances, such as herbs or spices. In China "kiu" was brewed from "sacrificial millet," a black variety which is possibly the oldest cereal known to man. The well-known "sake" of the Orient is a rice brew.

Africa and Asia

The Abyssinians still follow century-old, traditional rites in the preparation of "talla" or "merissa," as the Arabs call it. Barley is formed into a bread dough, roasted, and ground with raw cereals, boiled and fermented.

Malted "eleusina" is encountered all through the negro countries

as far as the Kaffirs in South and East Africa. This cereal, little known outside of Africa, is cultivated almost exclusively for brewing "bilbil," a perfectly clear, reddish brown, pleasantly bitter drink. Another beer made of eleusina is the "ana" of Equatoria, the Egyptian Sudan. In the French Sudan we find "dolo," brewed from millet, maize, and bananas and fermented with the aid of some roots, resulting in a palatable and spicy beverage.

The Sangonassis of the Congo have their "toko" or "pipi" and a rice beer flavored with hops which is brewed by the missionaries in Kimnerza. In East and Southwest Africa the women do all the brewing; there is "pombe," a millet beer, and also "omalofa" and "metabele," both made from kaffir corn. Today the municipal production of kaffir beer is licensed by the (English) government. Recent papers, published in the *Journal of the South African Chemical Institute*, report the results of a scientific investigation into the methods employed. "Kaffir" beer is produced by a partial fermentation of a millet malt gruel; it contains less than 3% of alcohol, approximately 0.75% of organic acids, and 7%–10% of solids. "Utshvala" is prepared from a mash of malted and unmalted grain, to which ground malted grain is added. Fermentation is allowed to start spontaneously and the mash is then strained. Such beers vary appreciably in composition. They probably are of high dietetic value since considerable quantities of vitamins B and C are present. Another variety, "marevu," has a pleasant acidity but contains no vitamin C.

The ancient Armenians kept their "busa" in large pots underground, sucking it up through long reeds. The Phaeonions in Macedonia brewed "zythos" from barley and "parabia" from millet, while the Arabians made a beer-like beverage from barley bread, raisins, honey, and spices.

When still inhabiting Asia, the Aryans made "sura" from panicum, a species of millet, by adding water, honey, curd, melted butter, and barley. This beverage was called "hura" by the western Aryans, living in Persia.

Brewing in Europe

During the first century B.C., Diodorus wrote of the Kelts or Gauls inhabiting the Spanish peninsula and the British Isles as follows: "Since the climate is too cold, their country produces neither wine nor olives. For this reason, they prepare for themselves a drink made of barley, the so-called 'cerevisa'"—"korina" in Irish.

In the 5th century A.D., Orosius reported on the Hispanians: "They prepare artificially out of the juice of wheat a warming beverage which they call 'celia.' Its fiery vigor is first awakened by the steep-

ing of the grain, which is then dried and worked into flour, whereupon it is added to the milk liquor and fermentation finally imparts a tart taste, as well as the heating and intoxicating quality." In the meantime the inhabitants of Spain had learned to brew beers of good keeping quality; they also used beer yeast for raising bread dough and for cosmetic purposes.

Beer was a favorite beverage in Britain and with all Germanic tribes. Tacitus wrote: "Their drink is a liquor prepared from barley or wheat brought by fermentation to a certain resemblance to wine." They also used oats. In Russia "quass" was made from rye and barley.

The first organized brewing system existed under Charlemagne. From this time dates the first authentic document mentioning brewing as a profession. The first reference to the application of hops is found in the writings of Saint Hildegard, who lived in the 11th century—"If thou desirest to make a beer from oats and hops. . . ." A beer recipe from the Tudor period in England calls for "10 quarters of malt, 2 quarters of wheat, 2 quarters of oats, with 11 pounds of hops."

Oatmeal stouts appear to have been quite popular in England and are still manufactured in Australia. The Bohemian beers were originally brewed from barley malt. Wheat or "weiss" beer was not heard of until the 15th century, and then the privilege of making it was reserved to the nobility and those who brewed for the authorities.

Native American Beers

According to a recent article in the *American Anthropologist*, entitled "Native American Beers," "there is ample evidence of a wide distribution of undistilled, alcoholic beverages, both beers and wines, in North as well as South America." Contrary to the old world, where barley, oats, millet, and similar grains were the most commonly used brewing materials, corn was the predominant cereal crop in the western hemisphere, from where it was introduced into other parts of the world. In both Americas numerous distinctly different strains of corn were grown, among them white, yellow, red, and blue varieties.

A most peculiar and to us little appealing procedure was used by the South American Indians to start fermentation, according to Karsten, author of *Civilization*. Instead of employing yeast or any other ferment, they chewed the corn or a baked cornmeal mush, believing that "the saliva, which shares the natural magic power of the whole body, was supposed to favorably influence the spirit that is active in the fermented drink."

The "chicha" of the South American Indians was made from boiled maize, chewed and fermented in large pots, covered with leaves, or in

cowhides spread over poles. This type of beer was known over wide territories, including Mexico, Guatemala, Ecuador, Peru, Chile, and the Andes. The drink of the Nicaraguan tribes was "mazamorro," made of a mixture of honey and ground corn. The natives of Juma roasted wheat grains over a charcoal fire until light brown in color, pulverized them, made a thick mash with water which was fermented. Of all grain-growing tribes only the Pueblos lacked fermented beverages, whereas many of their nomad neighbors had them.

Early American Brewing

Equally fascinating is a study into the historical background of our own brewing materials. Upon their arrival in the new world, the first settlers were faced with a lack of proper grains for malting. Therefore every newcomer was requested by decree to bring with him a certain amount of malt from England. Lack of grain, however, did not discourage people from brewing their favorite beer or ale, as this old colonial rhyme shows:

> "If barley be wanting, to make into malt,
> We must be contented and drink it no fault,
> For we can make beer to sweeten our lips,
> From pumpkins, and parsnips and walnut-tree chips."

For this reason, the suitability of Indian corn as a brewing material became one of the first research problems of cereal chemistry in this country. In his book, *The Advancing Front of Science*, G. W. Gray writes:

"It was in 1635 that the science obtained its first foothold in the New World. In that year John Winthrop, Jr., a young alchemist of the Massachusetts Bay Colony, visited England and obtained from the Crown a commission to develop certain native mineral resources. He was interested in the production of copper, glass, iron, lead, tar, and other 'chymicals,' including medicines. The Royal Society asked him to see if the grain, American maize, would produce beer. Winthrop tried it and brewed a 'pale, well-tasted middle beer.' He even did research on cornstalks and found that they yielded syrup sweet as sugar, a foretaste of the extensive corn syrup industry of today."

Ever since 1641 attempts were repeated to make malt from Indian corn, but never with any success. However, corn was used in other forms, and in 1662 Winthrop delivered a lecture before the Royal Society of London on the question of brewing with corn and in 1663 he treated the dignitaries with the first beer brewed with corn in Europe. It is reported that it met with great favor.

Both wheat and oats were grown by the early Dutch settlers in New York State for brewing purposes, while much malt was imported from European countries, particularly England, because no proper malting facilities were in existence here. The poorer classes, not being able to afford the costly imported barley malt, brewed their beer with molasses and bran. In New England sassafrass, boiled with roots or herbs, birch, spruce, or sassafrass bark, with pumpkins and apple parings, sweetened with molasses, maple syrup or beet tops substituted for malted grain in times of need. Poor grain crops occasionally were responsible for temporary prohibition periods; for instance, in 1641 Massachusetts prohibited the use of wheat for bread and malt in order to promote export.

In 1641 John Appleton of Massachusetts built what was probably the first malt house, as well as a brewery in Watertown, where he experimented with Indian corn. New England malt gained reputation and soon was "exported" to the other colonies, particularly Pennsylvania. The first patents of the manufacture of corn beers, brewed with unmalted corn, were granted to Alexander Anderson of Pennsylvania on January 26, 1801.

Malt adjuncts in various forms came on the market about 1850. Secret brewing procedures with raw grain were offered to brewers at exorbitant prices. The improperly prepared materials (patents to Fred Seitz, 1852–70) made from the whole kernel became rancid rapidly, imparting a bad flavor to the mash which had to be removed with bone black. This situation was improved by replacing yellow corn with white varieties and by removing the germ and husk. Satisfactory results, however, were not obtained until the whole problem was investigated scientifically, after which rapid progress was made.

"Cerealine," the first corn flake manufactured from shelled, deoiled, and ground corn by the application of heat and moisture, made its appearance on the market in 1883. "Frumentum," produced in strictly mechanical manner by the dry process, followed in 1891.

During the second half of the 19th century, the agricultural importance of brewing materials grew with the rapidly expanding brewing industry, and the cultivation of barley spread with the settlement of the Middle West, where it was grown with increasing success after 1860 in Ohio, Wisconsin, Iowa, and after 1870 in Minnesota, Nebraska, Utah, and later in the Dakotas and California. From 1861 on the Manchurian varieties were introduced in the Middle West.

However, it was not until the beginning of the 20th century that a barley-improvement program was organized by the Department of Agriculture in cooperation with the malting and brewing interests. At that time, there existed a controversy about the brewing value of

imported two-rowed barley malt as compared with American six-rowed varieties, leading to an extensive study of American barleys and malts, the results of which were published by the Department of Agriculture as Farmers' Bulletin No. 124, entitled "Chemical Studies of American Barleys and Malts," by J. A. LeClerc and R. Wahl.

BARLEY CORRELATION VALUES FROM SIX STATES, COMPARING CHEMICAL VALUES

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Barley has not been considered a commercial crop in Oklahoma, and many farmers and experiment station investigators seem to doubt the advisability of trying to increase the crop. Seeded in September or October, a very desirable winter pasture is provided, and the grain is harvested in May or June, before the heat of summer injures the quality. One or two species of small barleys are indigenous to Oklahoma, and the acreage of the cultivated crop is increasing each year. The object of this study has been to determine the relative commercial value of Oklahoma barley.

Specialists from California, Colorado, Michigan, Minnesota, and Wisconsin have very generously supplied us with samples of their most popular varieties to make a study of the comparative quality of Oklahoma selections with their barleys.

The weight of 1,000 kernels, the diastase, catalase, total nitrogen, and the hydrolyzable solids were considered most expressive of the commercial qualities of barley, as shown by Shellenberger and Bailey (1936). Catalase determinations have not been used extensively in determining the germinative power of barley, but Davis (1926) and Legatt (1929) indicate it to be a very effective method of estimating the germinating capacity of other seeds. Since the germination percentage has certain physiological limitations, catalase measurements have been substituted as a quantitative method of expressing the germinating power of the kernels.

The comparison of the results of the various estimations has been based upon multiple correlation values. Tabulated results eliminate the speculative relationships and make possible the reproduction of a similar study by other investigators.

Methods

The barley samples are listed in Table II, which includes the names of the varieties and the states from which they were obtained. Several of these have been used by other workers and are well known. One thousand kernels were counted out for each sample and weighed in grams. Samples of 100 grams were cleaned and ground with the finest plates of a small food chopper. This material was used for the following determinations.

Five-gram samples of the ground grain were used for diastase determinations, 2-gram samples for total nitrogen, 1-gram samples for catalase, and 2½ grams for hydrolysis. These were treated as directed in the Official Methods of the A. O. A. C. For catalase, the methods of Davis (1926) were used, with the exception of grinding.

The methods of Wallace and Snedecor (1931) were used in making the multiple correlation study. Tabulations were made after all estimations had been carefully repeated.

Results

Differences among barleys from the various experiment stations are shown in Table I. Kernel weights in Table I indicate that Cali-

TABLE I

AVERAGE VALUES OF BARLEY VARIETIES

State	Hydrolyzed solids	Total nitrogen	Catalase	Diastase	Wt. 1,000 kernels
	%	%	*cc.* O_2	*mg. maltose per 10 g.*	*g.*
Oklahoma	70.50	2.16	32.45	148.00	30.11
Wisconsin	70.40	1.78	27.76	112.00	26.60
California	67.40	1.74	21.72	72.80	41.48
Minnesota	67.20	1.96	32.12	126.80	29.14
Colorado	70.20	2.20	28.72	121.60	32.48
Michigan	71.40	1.64	29.70	144.00	36.40

fornia barleys are quite superior in plumpness and development of grains, but the other determinations for the California samples averaged very low. This is especially true with the enzymes. On the other hand, Michigan barleys have heavy, well-developed kernels, a high percentage of carbohydrate, low nitrogen, and high diastase. Oklahoma barleys are very poor in appearance, compared with those from the two states mentioned above, but the diastase and catalase content are unusually high. The first part of Table II shows the details for each variety.

Some interesting observations may be made of the results shown in

Table II. The highest percentage of hydrolyzable solids may be found in Michigan Winter barley, grown in Oklahoma. It also has a comparatively low total nitrogen content. It is to be noted that this barley is the highest yielding and one of the most promising types grown in Oklahoma and that it is not grown at the Michigan Experiment Station. Very near to it in yield and popularity is Missouri Beardless, high in diastase and hydrolyzable solids, but lacking in grain plumpness.

TABLE II

MULTIPLE CORRELATION TABLE OF FOUR INDEPENDENT VARIABLES, USING THE WEIGHTS OF 1,000 KERNELS AS THE DEPENDENT VARIABLE

Variety	Hydrolyzed solids	Total nitrogen	Catalase	Diastase	Wt. 1,000 kernels
	%	%	*cc.* O_2	*mg. maltose per 10 g.*	g.
Oklahoma					
1. Mich. Winter	77	1.8	33.9	94	35.4
2. Composite	73	2.0	27.3	150	34.5
3. Hero	68	2.2	36.0	68	33.3
4. Heron	69	2.0	33.9	126	32.0
5. Comfort	70	2.4	33.4	232	30.8
6. Black Smyrna	66	2.3	28.6	162	30.4
7. Missouri Beardless	71	2.2	31.0	204	23.2
8. Trebi	70	2.4	35.5	148	21.3
Wisconsin					
9. Trebi	75	1.7	26.2	120	34.6
10. Peatland	71	2.0	27.5	94	27.6
11. Wisconsin Beardless	69	1.7	30.0	124	25.0
12. Velvet	65	1.8	27.0	120	23.6
13. Oderbrucker	72	1.7	28.1	102	22.2
California					
14. Vaughn	65	1.6	22.2	68	48.3
15. Club Mariout	68	1.7	20.2	78	43.9
16. Tennessee Winter	69	1.6	19.1	66	42.2
17. California Coast	65	2.1	23.3	72	36.9
18. Hannchen	70	1.7	23.8	80	36.1
Minnesota					
19. Trebi	66	2.0	29.5	136	31.7
20. Wisc. No. 38	68	1.9	36.0	102	30.3
21. Velvet	71	1.9	29.4	110	28.9
22. Glabron	64	2.0	33.0	152	27.6
23. Manchuria	67	2.0	32.7	134	27.2
Colorado					
24. Club Mariout	72	2.2	26.9	110	26.6
25. Flynn	73	2.2	30.3	124	27.1
26. Velvet	68	2.4	31.6	122	34.6
27. Trebi	68	1.8	24.1	152	42.5
28. Colsess	70	2.4	30.7	100	31.6
Michigan					
29. Alpha	73	1.7	28.9	140	40.6
30. Trebi	74	1.4	26.6	150	40.1
31. Michigan Two-Row	72	1.8	38.8	160	36.4
32. Velvet	68	1.8	26.1	144	32.5
33. Wisc. No. 38	70	1.5	28.1	126	32.4
Sum	2297	63.9	959.7	4070	1071.4
Mean	69.6	1.94	29.1	123.3	32.5

TABLE II—*Continued*

SOLUTION OF NORMAL EQUATIONS

Variable	Hydrolyzed solids A	Total nitrogen B	Catalase C	Diastase D	Wt. 1,000 kernels X
		%	*cc.* O_2	*mg. maltose per 10 g.*	*g.*
A	——	−.1154	+.0516	+.1253	−.1363
B	——	——	+.3164	+.2235	−.3615
C	——	——	——	+.4169	−.5523
D	——	——	——	——	−.1364

By the normal-equation method R was found to be +.308, which is probably not significant.

The general appearance and weight of 1,000 kernel would indicate that the California barleys are quite superior to any other. The low total nitrogen would indicate that the proteins are not offensive in malt production. The low diastase and catalase content of these barleys is also noteworthy. The entire group of Michigan barleys have a correspondingly low total nitrogen content, a high diastase content, and very plump grains.

Trebi seems to be one of the most extensively grown experiment-station barleys. The hydrolyzable material is comparatively high in each group. In the Michigan group, Trebi has the lowest total nitrogen content of any selection and is high in hydrolyzable content, as well as in diastase. The fact that this variety is grown in five of the six states and that it has all the qualifications of a superior malting barley would indicate that Trebi is one of the best commercial barleys.

Dickson and others (1938) have the following statement to make regarding Trebi: "Trebi (C. I. No. 936) is a six-rowed, rough-awned variety and has large blue kernels. It is a selection from an imported lot of seed obtained by the United States Department of Agriculture from the south side of the Black Sea in 1905. The selection was made in 1909 by H. V. Harlan, of the United States Department of Agriculture, at the Minneosta Agricultural Experiment station. It was released for commercial production in 1918."

In the second part of Table II, in the solution of normal equations, line A, it may be observed that the correlation coefficients of nitrogen, catalase, diastase, and kernel weight are not especially significant, when compared with hydrolyzable solids. In line B, the comparison is made with the total nitrogen. It may be observed that the catalase and diastase have some significance and that the kernel weight is negative. The same is true in line C, in which we have a positive correlation of catalase and disatase, with a negative value for kernel weight. The multiple correlation, +.308, is probably not significant.

Summary

Multiple correlation data of the hydrolyzed solids, total nitrogen, catalase, diastase, and weight of 1,000 kernels are presented for barleys from six states. The coefficient of multiple correlation is of doubtful significance.

The correlations of 1,000-kernel weights with total nitrogen and diastase estimations are generally negative.

A general conclusion of the tabulation is that the higher the weight of grain is, the greater is the error of estimate.

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THE WHEAT-MEAL-TIME FERMENTATION TEST. III. EFFECT OF BRAN, PROTEASES AND ACTIVATORS ON THE "TIME" OF FLOUR[1]

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In a previous paper (Swanson, 1937) it was shown that the addition of bran to flour made the "time" different from that obtained on meal. Why the mixing of bran or shorts with the flour in the same proportion as these substances are found in the meal should make the time longer is a problem for study. Since it is the flour which is used in baking, it would seem more logical that the "time" test should be made on flour rather than the meal. There are also indications that the "'time" is influenced by substances present in the parts of the wheat kernel which are removed in the milling process. This situation raises a further ques-

[1] Contribution No. 58, Department of Milling Industry.
[2] Credit is due Glen West, student assistant, for able and painstaking work in performing the details of this time test.

tion as to the value of this test for indicating quality in flour. Since the effects of bran added to flour are very different from the effects of bran in the meal, it was thought worthwhile to make these tests on a wider scale than was done previously. Swanson and Dines (1939) showed that the proteases shorten the "time" and that this effect is much greater on long "time" than on short "time" wheats. Since bran and proteases have opposite effects, these substances were used in combination and protease activators were also tried in the present investigation.

The findings in this and previous papers are of significance because of the possible usefulness of this test to plant breeders, since "time" is apparently a variety characteristic. However, if the "time" can be lengthened or shortened by the addition of certain substances, especially those from the wheat itself, the question arises whether this is really a test of gluten quality or a test of the presence or absence of the substances which influence the "time." It is evident that before the figures obtained in this test can really be evaluated in respect to wheat quality, it is necessary to know the factors which determine the number of minutes which will elapse between the moment the doughball is put in the water and the breaking on the under side is observed.

Wheats Used

The two hard red winter wheats, and flours from the same, used in much of this investigation were Tenmarq, representing long "time," and Chiefkan, representing short or medium "time," of the crop of 1937. A 2,000-gram sample of each was milled, so as to get flour, bran, and shorts for these trials. The wheat meal was ground in a hammer mill to pass a ½-mm. sieve. The bran was also ground in the same manner. Additions to either meal or flour were mixed in beforehand so as to be thoroughly incorporated in the dough while this was mixed. The further details of performing the test have been given in the papers just cited.

The data obtained on the meals and flours from these two wheats are given in Table I. These figures were the averages of several trials on the meals and flours and may therefore be used for a general reference. It will be observed that the figures for the checks in the several tables given in this paper are not always the same as those in Table I. The variations are due to experimental errors and their magnitudes must be considered in making comparisons.

It is very evident that the "time" on the flours is much longer than on the meals and that the differences between the flours from these two varieties are less than between the meals. However, on the Tenmarq

TABLE I

TIME ON MEAL AND FLOUR FROM TENMARQ AND CHIEFKAN

Material	Tenmarq	Chiefkan
	Min.	*Min.*
Meal	121	59
Flour	132	111

flour the "time" is longer than on the Chiefkan flour. Hence these two wheats and their flours were suitable for these experiments.

It is much more difficult to obtain uniform readings on doughballs made from flours than on those made from meals, because the end point is more obscure at the flour doughball-water interface. For this reason the experimental error on flour is greater than on meal.

Effect of Adding Bran to Flour

In each case the bran from Tenmarq was mixed with Tenmarq flour and bran from Chiefkan mixed with the Chiefkan flour. Since the bran was ground on the same hammer mill as was used for grinding the wheat meal, the bran was of the same fineness but freer from endosperm than the bran flakes in the meal. The results obtained are given in Table II.

TABLE II

INCREASE IN "TIME" DUE TO ADDING BRAN TO FLOUR

Proportions in mixture	Tenmarq		Chiefkan	
	Total	Increase	Total	Increase
	Min.	*Min.*	*Min.*	*Min.*
15 g. flour (check)	137	—	110	—
14 g. flour+1 g. bran	182	45	148	38
13 g. flour+2 g. bran	194	57	174	64
12 g. flour+3 g. bran	205	68	168	58
11 g. flour+4 g. bran	—	—	171	61

It is evident that the effect of the bran when separated in the ordinary milling process is different from that of the branny material in the meal. This is not due to the granulation of the bran flakes, because the bran was ground in the same hammer mill as the meal. The longer "time" on flour in comparison with the meal might be explained on the basis of a denser gluten structure in the flour doughball. Mixing in the bran would dilute this gluten structure and hence the "time" should be shortened. Instead it is longer, and is still longer with the larger amounts of bran which would dilute the gluten structure all the

more. It seems therefore that the lengthening is due to some substance in the bran which inhibits the factors which cause the disintegration of the doughball. However, this inhibition is not effective when the bran material is in the meal. The meal differs in two important respects from the mixture of bran and flour. In the meal the particles of endosperm are coarser and the germ is also present. That the granulation alone does not account for all this difference was shown in the following trials.

Effects of Granulation

Granulation of the meal may be affected by tempering so as to have a softer endosperm and also by regrinding the meal first ground in the hammer mill. The wheat was tempered at 14%, 16%, and 18% moisture. The regrinding was done by passing the meal first ground in the hammer mill, through the corrugated rolls of the Allis mill set as for the fourth or last break. The material was then sifted over 30 grits gauze and the throughs ground on the smooth rolls set as for the third middlings reduction. This was then sifted over the 10XX and the overs were again ground between the smooth rolls set as for the last reduction. This would reduce the endosperm to almost the same fineness as in flour. The results from these various grindings are given in Table III.

TABLE III

EFFECT OF GRANULATION OF THE MEAL ON TIME

Treatment	Tenmarq	Chiefkan
	Min.	*Min.*
Ground once in hammer mill	121	59
Meal reground in Allis mill	137	82
Tempered at 14%, ground once in hammer mill	129	64
Tempered at 16%, ground once in hammer mill	117	65
Tempered at 16%, meal reground in Allis mill	139	92
Tempered at 18%, ground once in hammer mill	118	61
13 g. flour+2 g. ground bran	194	174

While the "time" is increased by the finer granulation, it does not become as long as when bran is mixed with the flour.

Effects of Shorts and Germ Stock

Shorts contain bran material in a finely pulverized condition and also considerable endosperm from near the bran coat. The germ stock was a mill stream containing about 10% germ material mixed with the shorts in the usual milling process. The shorts were mixed with the

flour in a manner similar to that of bran so that the shorts from Tenmarq wheat were mixed with the Tenmarq flour and the same procedure was used with Chiefkan. The results obtained are given in Table IV.

TABLE IV

INCREASE IN "TIME" DUE TO SHORTS AND INCREASE OR DECREASE DUE TO GERM STOCK

Material	Tenmarq		Chiefkan	
	Total	Increase or decrease	Total	Increase or decrease
	Min.	*Min.*	*Min.*	*Min.*
15 g. flour (check)	137	—	110	—
14 g. flour+1 g. shorts	183	46	136	26
13 g. flour+2 g. shorts	221	84	180	70
12 g. flour+3 g. shorts	257	120	183	73
14 g. flour+1 g. germ stock	180	43	145	35
13 g. flour+2 g. germ stock	160	23	70	−40
12 g. flour+3 g. germ stock	79	−58	54	−56
10.5 g. flour+2.3 g. bran+2.2 g. shorts	275	138	220	110

A comparison of the figures in Tables IV and II shows that shorts lengthen the "time" on Tenmarq as much as bran, or even more. Shorts contain endosperm material from near the bran coat similar to fourth-break flour. This flour has a longer time than the other mill-stream flours (Table VI). This together with the possible inhibiting effect of bran would account for the longer time obtained with the mixture of Tenmarq shorts and flour. The increase in time from one gram of germ stock which would contain more germ than present in 15 grams of meal from the whole wheat is probably due to the inhibiting effect of the branny material and to endosperm material similar to that in the shorts. The shortening of the "time" by larger amounts of germ stock may be due to a protease in the germ or to the phosphatides present in the germ, or possibly both.

Influence of the Germ

That the germ does have a shortening effect on "time" was further shown by making the determination on meal from which the germ had been removed, in comparison with meal containing double the amount of germ naturally present and meal ground from the whole kernel. The Tenmarq and Chiefkan used in this trial were not from the same lots as those used in the other trials reported in this paper, but the time of the former had been found to be twice that of the latter. A small lot of each was used for dissection and the germ end of each

kernel was cut off with a sharp scalpel while the kernel was held by tweezers under a four-inch magnifying glass. After this separation the portions were weighed and ground in a coffee mill. Whole-wheat portions were also ground in the same mill so as to minimize differences due to method of grinding. The data obtained in this trial are found in Table V.

TABLE V

EFFECT OF GERM ON THE "TIME"

Material	Tenmarq		Chiefkan	
	Percent of kernel	Time	Percent of kernel	Time
		Min.		*Min.*
Whole meal	100	82	100	41
Brush ends	83	118	81	62
Germ ends	17	—	19	—
Whole meal+germ	—	51	—	32

The amounts obtained from the germ ends were too small for a separate determination. The proportions in whole meal + germ were the same as obtained in the dissection. It is very evident from the data that the "time" on meal from which the germ has been removed is considerably longer than on meal from the whole kernels. It is also evident that when germ is added so that the meal contains twice as much germ as originally present, the time is considerably shorter. That is, when the germ is removed the "time" is longer and when added it is shorter. Thus the germ contains some substance, probably a protease or a phosphatide, which greatly influences the "time."

That there is a variation in "time" on material obtained from different parts of the kernels was shown by making the determination on various mill stocks. The samples described in Table VI were obtained from milling locally grown Tenmarq wheat. All the products except the flours were reground in the hammer mill so as to have the usual degree of fineness. It is very evident that too much branny material such as is present in second- and third-break stock weakens the gluten structure and hence shortens the "time." The various flours as well as the middlings, except the first tailings, all have longer "time" than the wheat meal when bran is added to the flour; the time, however, is longer than on the meal. Regrinding the middlings would reduce them to nearly the same fineness as the flour. The shorter "time" on the first tailings is probably due to the presence of the germ, most of which goes into this stream. The sizings also contain considerable germ, which probably causes the shorter time. When the germ material is

TABLE VI

TIME ON WHEAT AND VARIOUS MILL STOCKS

Material	Time
	Min.
Wheat above first-break rolls	89
Stock above second-break rolls	67
Stock above third-break rolls	33
Sizings, above rolls	82
First middlings above rolls	100
Third middlings above rolls	124
Fifth middlings above rolls	116
First tailings above rolls	84
First-break flour	105
Fourth-break flour	139
Sizings flour	117
Third-middlings flour	102
First-tailings flour	121
Straight flour	124
12 g. straight flour+3 g. bran	132

removed by sifting, as is the case with the sizings and first-tailings flour, the time corresponds in length to that of other flours.

That the germ has a tendency to shorten the time thus appears from three trials: (1) the addition of germ stock to flour, (2) the increase in the germ content of the wheat meal, (3) the shorter time obtained on mill streams which contain the germ as compared with those which do not.

Effect of Water-Extracted Bran

It seems that the lengthening of the time by bran and shorts when mixed with flour is due to some inhibiting substance. To see if this can be removed by water extraction, 35 g. of bran of each variety was stirred up in 420 c.c. of water and allowed to soak over night. This was then filtered on linen cloth, washed with several portions of water, and drained between washings. The bran was then spread out in a thin layer until air dry, and then ground in the hammer mill. The results obtained by mixing portions of this extracted bran with flour in making the doughballs are given in Table VII.

The results obtained indicate that all of the active substance was removed by the water extraction from the Chiefkan bran and only partly from the Tenmarq bran. That is, after water extraction the Chiefkan bran acts merely as so much inert material. In the previous investigation (Swanson, 1937) it was shown that inert substances such as paper pulp and alundum powder had no effect on the "time" of flour. Whether a more thorough extraction of the Tenmarq bran might have made this inert also was not determined, but it is evident that the bran

TABLE VII

EFFECTS OF WATER-EXTRACTED BRAN

Material	Tenmarq		Chiefkan	
	Total	Increase	Total	Increase or decrease
	Min.	*Min.*	*Min.*	*Min.*
Flour alone, check	132	—	111	—
14 g. flour+1 g. extracted bran	157	25	110	−1
13 g. flour+2 g. extracted bran	174	42	112	1
12 g. flour+3 g. extracted bran	164	32	103	−8

from the two wheats behaved differently after the process of water extraction.

Effect of Water Extract of Bran

To determine what effect the water extract of bran has on "time," 50 g. of bran of each variety was placed in 200 c.c. of water and allowed to soak over night. This proportion was used because it requires about four grams of water to wet thoroughly one gram of bran and leave enough liquid that can be used for the tests. Four c.c. of the extract thus represented one gram of bran. The soaked bran was placed on linen cloth and as much of the extract as possible squeezed out. This extract was used in amounts and with the results given in Table VIII.

TABLE VIII

DECREASE IN "TIME" DUE TO WATER EXTRACT OF BRAN

Material	Tenmarq		Chiefkan	
	Total	Decrease	Total	Decrease
	Min.	*Min.*	*Min.*	*Min.*
Flour alone	132	—	111	—
15 g. flour+2 c.c. extract (½ g. bran)	93	39	88	23
15 g. flour+4 c.c. extract (1 g. bran)	116	16	88	23
15 g. flour+6 c.c. extract (1½ g. bran)	124	8	93	18
15 g. flour+8 c.c. extract (2 g. bran)	116	16	94	17

Since the extract of bran decreases the time of both varieties, there seems to be an activating substance which is present in the water extract of bran. It should be noted, however, that the progressively larger amounts of extract do not have a proportionally greater effect. Thus the extract of bran added to flour in making the doughball has effects opposite to that of untreated bran. The soaking over night apparently stimulates some activator, or phosphatides may be hydrolyzed. In a

previous investigation (Swanson, 1937) an increase in time was obtained from the use of the extract of bran but the method of making the extract was not the same in the former trial.

Effect of Pepsin on Mixtures of Flour and Bran Materials

That pepsin has a distinct effect in shortening "time" has been shown by Swanson and Dines (1939). It was desired to learn whether pepsin would overcome the retarding effect of the bran. Hence trials with bran, extracted bran, and bran extract were made using 2 mg. of pepsin for each 15 g. of mixture. The results obtained are given in Table IX. The figures obtained without pepsin are repeated from the preceding tables for comparison.

TABLE IX

EFFECT OF PEPSIN ON MIXTURES OF FLOUR AND BRAN MATERIAL

Material	Tenmarq			Chiefkan		
	Without pepsin	With pepsin	Decrease	Without pepsin	With pepsin	Decrease
	Min.	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*
Wheat meal	121	27	94	59	32	27
Flour	132	67	65	111	62	49
Untreated bran						
14 g. flour+1 g. bran	182	58	124	148	51	97
13 g. flour+2 g. bran	194	80[1]	114	174	49	125
12 g. flour+3 g. bran	205	38	167	168	39	129
Extracted bran						
14 g. flour+1 g. ext. bran	157	72	85	110	48	62
13 g. flour+2 g. ext. bran	174	43	141	112	44	68
12 g. flour+3 g. ext. bran	164	30	134	102	37	65
Bran extract						
15 g. flour+2 c.c. ext.	93	52	41	88	78	10
15 g. flour+4 c.c. ext.	116	59	56	88	80	8
15 g. flour+6 c.c. ext.	124	62	62	93	64	29
15 g. flour+8 c.c. ext.	116	68	48	94	70	24
15 g. flour+2 c.c. ext.[2]	93	62	31	88	79	9
15 g. flour+4 c.c. ext.	116	66	50	88	78	10
15 g. flour+6 c.c. ext.	124	69	55	93	77	16
15 g. flour+8 c.c. ext.	116	76	40	94	77	17

[1] Since this figure is so far out of line, an experimental error is probable.
[2] Four mg. pepsin was used in the last four trials.

When pepsin is added together with the bran the time is much decreased as compared with flour alone and the decrease is greater with the larger amounts of bran. This is in contrast with the use of bran alone, which when mixed with flour increased the time and the increase

was greater with the larger amounts of bran. While bran alone retards the time for the breaking of the doughball, the bran plus pepsin shortens the time more than does pepsin alone. Also when both bran and pepsin are added the time on both Tenmarq and Chiefkan are practically the same. The pepsin, together with the extracted bran, shortens the time very markedly and with the largest amount of bran there is no essential difference between Tenmarq and Chiefkan flours. The effect of the bran extract when used with pepsin is less than that of the extracted bran, but here also the differences in time on Chiefkan and Tenmarq flours are negligible. Using 2 or 4 mg. of pepsin seems to make very little difference on the results.

Effect of the Protease Activator Cysteine-Monohydrochloride

In the previous paper (Swanson and Dines, 1939) it was shown that the protease activator, anhydrous cysteine-monohydrochloride, decreased the time on the meal from a long "time" wheat but not on a short "time" wheat. The trials with the activator were repeated and it was used in 2 and 4 mg. amounts for each 15 g. of flour or mixture. The results obtained with various combinations are given in Table X.

TABLE X

EFFECT OF THE PROTEASE ACTIVATOR CYSTEINE-MONOHYDROCHLORIDE

Material	Tenmarq			Chiefkan		
	Without C-M HCl	With C-M HCl	Decrease	Without C-M HCl	With C-M HCl	Decrease
	Min.	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*
15 g. meal	121	89	32	59	39	20
15 g. flour	132	113	19	111	100	11
14 g. flour+1 g. bran	182	141	41	148	75	73
13 g. flour+2 g. bran	194	181	13	174	121	53
12 g. flour+3 g. bran	205	175	30	168	121	47
Extracted bran						
14 g. flour+1 g. ext. bran	157	117	40	110	88	22
13 g. flour+2 g. ext. bran	174	94	80	112	86	26
12 g. flour+3 g. ext. bran	164	97	67	103	77	26
Bran extract						
15 g. flour+2 c.c. extract	93	80	13	88	84	4
15 g. flour+4 c.c. extract	116	85	31	88	93	+5
15 g. flour+6 c.c. extract	124	91	33	93	101	+8
15 g. flour+8 c.c. extract	116	101	15	94	123	+29
15 g. flour+2 c.c. extract [1]	93	82	11	88	88	0
15 g. flour+4 c.c. extract	116	90	26	88	96	+8
15 g. flour+6 c.c. extract	124	93	31	93	107	+14
15 g. flour+8 c.c. extract	116	103	13	94	109	+14

[1] Four mg. C-M HCl added in the last four trials.

The cysteine-monohydrochloride reduced the time in all cases except with the bran extract of Chiefkan, which produced an increase, but the observed reduction in time was much less than with pepsin. The reduction in time for flour alone was less than for either the meal or the flour plus bran, which indicates that flour contains very little of the substance which may be stimulated by the protease activator. With the untreated bran the reduction in time was greater for Chiefkan than for Tenmarq. The bran extract had a different effect than the extracted bran. The nil effect or increase in time when the activator was used with Chiefkan bran extract indicates that this contained no substance which could be activated or that it even contains an inhibitor. Thus these varieties behave differently both towards the activator and towards the protease pepsin.

Effect of Concentration of HCl at the Water-Dough Interface

That protease activity is influenced by the hydrogen-ion concentration of the solution is well known. Since the disintegration of the doughball starts at the water-dough interface, the time should be influenced by that pH which is near optimum for the protease. To try the influence of various concentrations of HCl on time, different amounts of a 0.1N solution of HCl were added to the water in the glasses. The results of combinations of acid and water in the glasses are shown in Table XI.

TABLE XI

Effect of the Concentration of HCl at the Dough-Water Interface

Combination	Tenmarq	Chiefkan
	Min.	*Min.*
200 c.c. water	121	59
190 c.c. water+10 c.c. 0.1N HCl	100	49
180 c.c. water+20 c.c. 0.1N HCl	59	41
160 c.c. water+40 c.c. 0.1N HCl	46	43

It is very evident that the concentration of HCl at the dough-water interface has a distinct effect on the time and that the differences between the two wheats tends to disappear as the acidity increases. Whether this was due to protease stimulation or to the action of the acid in weakening the gluten cannot be determined from this trial.

Summary

The presence of wheat germ has a significant influence on "time." The time was shortened: (1) by adding to flour a mill stream rich in germ; (2) by the presence of germ in mill streams such as tailings and

sizings; and (3) by doubling the germ content of the meal. The time was lengthened by removing the germs from the kernels before these were ground into a meal.

The addition of bran or shorts increased the time on flour from both a short and a long "time" wheat. When the bran was extracted with water, it increased the time on a long but not on a short "time" wheat. The water extract of bran decreased the time on both wheats. While a finer granulation has a lengthening effect on time, this alone does not explain the longer time on mixtures of flour and bran.

When pepsin was added to mixtures of flour and untreated bran, to water-extracted bran, and to bran extract respectively, the time was shorter than on flour alone, and there were no significant differences between the long and the short "time" wheat flours.

When the activator cysteine-monohydrochloride was added to the mixture of flour and bran, both natural and water extracted, the time was shortened, but not as much as with pepsin. When added with the bran extract, this activator shortened the time of a long "time" but not of a short "time" wheat flour. In the case of the latter, the time was increased.

The time was shortened by adding HCl to the water in the glasses so as to increase the hydrogen-ion concentration at the dough-water interface. Since the decrease was greater for the long-time than for the short-time wheat, the differences between the two tend to disappear.

It seems from this and previous investigations that the time is influenced by at least four factors: proteases, protease inhibitors and activators, and gluten quality. These factors may remove the differences between long and short "time" wheats. However, even after some treatments differences still persist. Further investigations are in progress.

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ABSORPTION-MOBILITY RELATIONSHIPS IN WHEAT-FLOUR DOUGHS[1]

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In earlier discussions of the "viscosity," or its reciprocal, the mobility of dough, it has been intimated at times that the proportion of water for the production of optimum quality of bread could be determined directly from such mobility measurements. Thus it has been suggested that doughs mixed to a certain definite mobility, as indicated by such an instrument as the farinograph, would then contain the requisite proportion of water for baking, regardless of the class, grade, and composition of the flour used in producing the dough. In order to test this hypothesis a series of studies were undertaken with flours of widely varying composition.

Nine flours falling into the three classes of so-called "strong," "medium strength," and "weak" were used. The "strong" flours were milled from hard red spring wheat, two of them being bakers' patents, the third a clear-grade flour. The "medium strength" flours included a spring wheat bakers' patent, a southwestern hard red winter wheat bakers' patent, and a southwestern hard red winter wheat bakers' patent of the 1935 crop. The "weak" flours were soft red winter wheat cake flours, two of which were short patents, while the third was of longer extraction. Their description and crude protein content are recorded in Table I.

Mobility was measured with a Brabender farinograph in consistency units, using 300 g. of the flour and sufficient distilled water to equal the particular absorption desired.

The Hobart-Swanson dough mixer was used in these tests. For determining the optimum absorption the doughs were mixed two minutes. The dough formula included 200 g. flour, 2 g. salt, 5 g. sugar, 6 g. yeast, and sufficient distilled water to equal the absorption desired. The doughs were brought out of the mixer at 30°C. Immediately on removal from the mixer they were folded in the hands ten times to insure that any inequalities of mixing, if present, would be more evenly distributed throughout the doughs. The doughs were then scaled into two portions of 160 grams each. This practice was followed for several reasons. In commercial practice a flour is judged by a loaf from a

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TABLE I

DESCRIPTION OF THE NINE FLOURS

Biochem. No.	Flour	Crude protein (N×5.7)
		%
	Weak Flours	
17890	Soft red winter wheat flour, short extraction	8.30
17891	Soft red winter wheat flour, longer extraction	9.58
17934	Soft red winter wheat flour, short extraction	8.30
	Medium-Strength Flours	
17892	Spring wheat, bakers' patent	13.18
17893	Southwestern hard red winter wheat, bakers' patent	12.65
17894	Southwestern hard red winter wheat, bakers' patent 1935	11.48
	Strong Flours	
17895	Bakers' spring wheat clear	15.10
17896	Bakers' spring wheat patent	15.08
17935	Bakers' spring wheat patent	15.10

scaled weight of dough, not from a scaled weight of flour. It also avoids inequalities due to unequal-sized doughs as absorption is varied.

Each piece was folded in the hands ten times to round it up and provide it with a smooth, tight, surface film. The doughs were fermented in porcelain-coated, round-bottom bowls which were lightly greased with a hydrogenated shortening to make handling of the doughs during punching more uniform. Were all doughs mixed to the same degree of mobility this would not be necessary, but, where mobility varies from extremely soft and sticky to very stiff doughs, an error would be introduced because of unequal handling. This error, reflected in the loaf, should not be charged to absorption but to handling, and the procedure followed was designed to reduce this error to a minimum.

Fermentation was conducted in a thermostat provided with humidity and temperature control. Weak flour doughs were fermented 1½ hours with a punch of ten folds one hour after mixing. Thirty minutes later they were hand-molded on a piece of heavy canvas belting. A small quantity of dusting flour was used to prevent the doughs from sticking to the belting. Doughs made of "medium strength" and "strong" flours were fermented three hours. They were punched with fifteen folds by hand after 105 minutes of fermentation and again with ten folds after 50 minutes of fermentation following the first punch. In 25 minutes they were hand-molded as previously described for "weak" doughs and all were panned in lightly greased, high-sided, black iron pans.

All doughs were proofed for 55 minutes at 30°C. in the fermentation cabinet and then baked for 25 minutes at 230°C. in an automatically

controlled, electric oven on revolving platforms. Humidity in the oven was supplied by an open bowl of water at the level of the rotating platforms.

The loaf type, according to the Blish method, was assigned each loaf as well as a score on a zero-to-ten basis. Thirty minutes after removal of the loaf from the oven its volume was determined by means of the Werner loaf-measuring apparatus. On the following day the loaves were cut open and scored for grain and texture. Quality score (Q.S.) was calculated by the following formula: $Q.S. = 0.1\ (L.V. - 200) + (L.T.S. + 2G + T)$.

L.V. = loaf volume in cc.
200 represents average original volume of dough in cc. when panned
L.T.S. = loaf type score
G = score for grain
T = score for texture

The approximate absorption of each flour was predetermined by means of the Brabender farinograph. A charge of 300 grams of flour and enough distilled water to bring the peak of the development curve to 550 units of consistency were first used. With this determination as a guide, the baking of the samples proceeded as previously described, the absorption being varied by one percent intervals. Doughs ranged in mobility from very stiff to very soft, or until loaf volumes showed an increase from low, through a maximum, and then to a definitely lower figure.

After the flours had all been baked and Q.S. or quality score calculated, a curve was plotted for each flour with Q.S. on the vertical axis and absorption on the horizontal axis. These curves are shown in Figure 1. It will be noted that "medium strength"

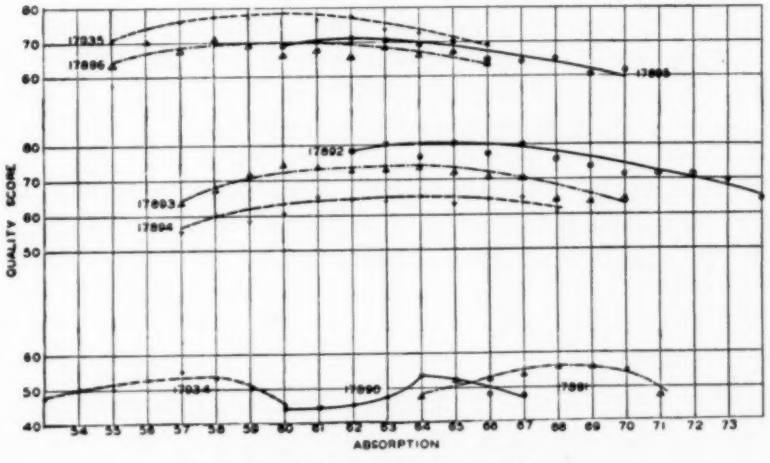

Fig. 1. Relationship of absorption to quality score.

and "strong" flours show no single definite peak such as is shown by the "weak" flours. In such cases an absorption was chosen, arbitrarily, as the optimum, from near the middle of the high part of the curve. As might be expected, these curves show that absorption is not an extremely critical factor in producing bread from strong bread flours. Such is not true when the "weak" flours are considered.

After the optimum absorption on each flour had thus been determined, the mobility curves of each were recorded on the farinograph for a 30-minute period. The absorptions used in these mobility studies were the optimum as determined by baking and one and two percent below and above the optimum, five levels in all. The effect of increments of one percent absorption on the mobility of the flour being studied and the different effects on flours of different "strengths" were thus disclosed. These data are recorded in Table II.

Following the mobility studies, using the farinograph, the flours were again baked, using the optimum absorption previously determined by the preliminary baking. In this second baking study the procedure was varied by the use of four periods of mixing time, 1, 2, 3, and 5 minutes, respectively. Each dough was scaled into 160-gram portions and three such portions were fermented for 1½, 2¼, and 3 hours, respectively. Thus each flour was accorded three fermentation treatments with each of the four states of mobility as imposed by the Hobart-Swanson mixer.

The purpose of this second study of the nine flours, by baking methods previously described, was to transfer the physical properties of the doughs at different stages of mechanical development in the farinograph to a form, the baked loaf, where the effect of these physical properties on loaf volume, loaf type, grain, texture, and loaf quality could be accurately measured. These stages of mixing development are equivalent to 6, 12, 18, and 30 minutes respectively in the farinograph.

From a study of the measurements of loaf volume and calculations of loaf-quality scores on these twelve loaves in comparison with the farinograph curves showing rate and degree of development (see Table III) and time, rate, and degree of slackening of dough, one is able to determine whether the information given by the farinograph alone is sufficiently exact and accurate for the purpose.

TABLE II

EFFECTS OF ABSORPTION ON MOBILITY

Flour	Absorption	Time to peak	Consistency units at peak of farinograms	Average difference in consistency units per 1% water	Consistency units after 30 min. mixing	Average difference in consistency units per 1% absorption
	%	*min.*				
17935	62	7½	670		490	
"	61	7	710		510	
"	60	6½	730		525	
"	59	6	770		535	
"	58	5	810	35	550	15
17896	63	6½	690		505	
"	62	6	710		510	
"	61	6	750		530	
"	60	4½	800		550	
"	59	4½	850	40	565	15
17895	64	6	610		425	
"	63	5	645		440	
"	62	4½	690		460	
"	61	5	725		470	
"	60	4½	760	39	485	15
17894	66	12	500		405	
"	65	11¾	515		420	
"	64	6	550		430	
"	63	5	570		450	
"	62	5	600	25	465	15
17893	66	10½	540		400	
"	65	10	550		410	
"	64	10½	570		420	
"	63	6½	605		445	
"	62	6	620	20	450	12½
17892	67	11½	455		340	
"	66	11	470		345	
"	65	11¾	510		370	
"	64	12	530		400	
"	63	6	580	31	420	20
17934	59	8	350		270	
"	58	8½	370		290	
"	57	8¾	390		305	
"	56	9	400		320	
"	55	9½	420	17½	335	16
17891	70	6½	325		220	
"	69	6	340		220	
"	68	5¾	355		225	
"	67	6	420		240	
"	66	6	425	25	280	15
17890	66	6	340		235	
"	65	6	340		230	
"	64	6	365		240	
"	63	6½	410		250	
"	62	7	425	22½	280	11

TABLE III

LOAF QUALITY SCORES

Flour No.	Fermentation periods—hours: 1½ Minutes mixed 1	1½ 2	1½ 3	1½ 5	2½ 1	2½ 2	2½ 3	2½ 5	3 1	3 2	3 3	3 5
17895	63.5	68.5	71.7	64.0	60.5	70.2	65.7	53.2	60.0	67.5	68.0	54.2
17896	79.5	80.5	72.0	64.5	75.0	73.7	67.5	52.7	66.7	68.7	62.0	52.7
17935	84.2	85.0	86.0	69.5	86.0	78.0	70.0	56.5	77.5	75.5	64.7	55.7
17892	66.2	77.5	79.0	73.2	63.2	65.5	66.7	62.0	57.7	64.7	63.5	60.2
17893	71.7	77.5	62.2	69.5	65.5	66.2	66.7	64.5	58.2	64.7	63.2	60.0
17894	64.7	59.7	69.5	64.7	58.0	61.0	63.5	61.5	57.0	60.5	65.7	62.0
17890	46.7	46.7	55.0	50.5	40.0	41.0	46.0	41.5	37.2	40.0	40.5	43.5
17891	43.0	45.5	52.5	50.0	42.5	45.0	45.7	43.0	39.0	40.5	42.2	40.7
17934	48.0	50.7	51.7	52.0	41.0	46.7	46.7	45.0	40.0	43.0	42.5	41.5

Discussion

If doughs prepared with the nine flours were mixed to a uniform consistency of 550 farinograph units, the proportion of water recorded in the second column of Table IV would be required. As a matter of fact, the optimum absorption, as determined by baking tests, was often

TABLE IV

ABSORPTIONS DETERMINED BY BAKING TESTS VERSUS ABSORPTIONS AS DETERMINED BY "STANDARD CONSISTENCY" METHODS

Biochem. No.	Absorption at 550 consistency units	Optimum absorption (by baking)	Consistency units at optimum absorption
	%	%	
17890	59	64	365
17891	61.3	68	355
17934	55	58	390
17892	66	65	510
17893	66.7	64	570
17894	65.7	64	550
17895	71	62	690
17896	71	61	750
17935	71	60	730

quite different from that required to produce dough of a standard consistency at the time of the original mixing. This is evident on comparing the data in column 3 of Table IV with those in column 2. It accordingly followed that the consistencies of doughs which baked best

when prepared from the nine flours were not uniform, as is evident from the data recorded in column 4 of the same table. The "weak" flours had low absorptions at 550 consistency farinograph units, the "medium strength" flours had a higher absorption, approximately 66%, and the "strong" flours had a very high absorption of 71%. This is in general agreement with the commercial practice of assigning high absorptions to "strong" flours and low absorptions to "weak" flours.

The "optimum baking quality" doughs prepared from "weak" flours were found to have an average absorption of 63.3% instead of 58.4% based upon the farinograph consistency, a gain of almost 5%; "medium strength" flours 64.7%, instead of 66.8%, a loss of about 2%; and "strong" flours 61% instead of 71%, a loss of 10%. Thus the fallacy of using a single mobility value for a wide range of flours in determining their absorption by the farinograph is apparent.

The second mobility studies, using the farinograph, with the optimum absorption of each flour, showed that mobility at optimum absorption is, on the average, approximately 370 for "weak" flours, 543 for "medium" flours, and 723 for "strong" flours. In the same class of flours, the longer-extraction flours had greater mobility or lower consistency-unit value at optimum absorption than the shorter extraction flours.

Summary and Conclusions

The relationship between optimum absorption and mobility as measured with the farinograph at that absorption was determined on nine flours, three of which were in each of "weak," "medium strength," and "strong" classes.

It was apparent that the mobility of a flour at optimum absorption is characteristic of that particular flour and that it varies widely between flours differing considerably in "strength."

FURTHER STUDIES UPON THE RELATIVE MACARONI-MAKING QUALITY OF A NUMBER OF DURUM WHEAT VARIETIES[1]

D. S. BINNINGTON and W. F. GEDDES[2]

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The present paper represents an extension of the studies upon the quality of durum wheat varieties initiated in 1934 by Binnington and Geddes (1937). Since that time, certain additional quality tests have been developed and a more extensive range of samples studied. It has thus been found feasible to draw more definite conclusions than were possible with the limited number of samples available in the earlier studies. The accumulation of a larger volume of data has also made possible statistical examination of the relations between certain of the more important quality factors.

As the authors have previously indicated, the literature in this field is extremely meager, being confined largely to a brief description of the agronomic, milling, and macaroni-making characteristics of the standard varieties contained in the "Dictionary of Spring Wheat Varieties" published by the Northwest Crop Improvement Association (1933). More recently, however, Fifield *et al.* (1937) have published the results of studies extending over a five-year period, which represent a valuable contribution to our knowledge of this subject.

Materials and Methods

The wheats employed in these studies were all experimentally grown on one-sixtieth-acre plots during the years 1935, 1936, and 1937. In 1935, samples were available from Morden, Manitoba, only; in 1936, Morden, Brandon, and Winnipeg; and in 1937 these three stations were supplemented by Melita, Manitoba, and Indian Head, Saskatchewan. Official grades and weights per Imperial bushel were secured upon all samples, which were milled into semolina and processed into macaroni by the technique outlined by Binnington and Geddes (1936). Protein and carotene determinations were made on the wheats and semolinas by the official A.A.C.C. methods, using the spectrophotometric procedure for the carotene determinations; water-saturated n-butyl alcohol, as described by Binnington, Sibbitt and Geddes (1938) was employed

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as a solvent for the later groups of samples. Where results were secured by use of the older naphtha-alcohol solvent, conversion to a butyl-alcohol basis has been made by use of the formulas outlined by Binnington and Geddes (1939). All samples for protein and pigment determinations were prepared by grinding in a Wiley mill fitted with a ½-mm. sieve, and all results are expressed on a 13.5% moisture basis.

Breaking strength of macaroni and tenderness of the cooked product were measured by the procedures outlined by Binnington, Johannson, and Geddes (1939), and color analyses of macaroni were also conducted by the methods described by these investigators, employing a modified Bausch and Lomb Type H. S. B. Color Analyzer with suitable Munsell discs.

Experimental Results

In view of the large number of samples studied, individual data are not presented in all cases, and the results obtained have accordingly been summarized by years. These results are detailed in Tables I, II, and III, and a general summary of means for all years and stations is given in Table IV. The listing of the samples is in the order of decreasing mean color score as shown in Table IV.

Macaroni color may be justifiably considered as the major measurable quality characteristic, and experience gained in this laboratory indicates that the so-called " computed " or " single figure " color score is a very reliable quantitative index of visual color. It is a difficult matter to establish rigid limits for this value, however, as the general level of durum wheat quality varies widely from year to year, depending upon the magnitude and nature of the degrading factors present, and a value that would be considered as normal for one crop may not be attainable at all in the following year. In a general way, however, a level of 18 units for this value would appear to represent a normal lower limit. In years where the general color level is low, this might be reduced to 17.5 units, but samples ranking below this value are definitely unsatisfactory.

Utilizing the above values as criteria, an inspection of the data in Tables III and IV indicates that only three varieties, namely Arnautka, Mindum, and Akrona, can be considered as consistently yielding macaroni of satisfactory commercial quality over a period of years; the latter variety can only be classed as " borderline." This conclusion is confirmed by an examination of the color analysis data for the individual stations presented in Table V. Using 18 units of color score as the critical level, seven out of eight samples of Arnautka were above this value, Mindum five out of eight, and Akrona, Pelissier, and Nodak

TABLE I

WEIGHT PER BUSHEL, GRADE, AND SEMOLINA YIELD
Comparison of Mean Values—All Stations—Years 1935, 1936, and 1937

Variety	Weight per Imperial bushel (cleaned wheat)			Official grade [1]			Semolina yield		
	1935	1936	1937	1935	1936	1937	1935	1936	1937
	lbs.	*lbs.*	*lbs.*				%	%	%
Arnautka	59.0	62.5	63.2	4.0	3.0	2.4	26.4	30.4	31.2
Mindum	61.0	62.7	64.0	3.0	3.0	2.0	27.4	30.3	34.8
Akrona	56.0	62.3	63.9	4.0	3.3	2.4	24.5	29.1	32.0
Iumillo	—	62.6	63.9	Red	Red	Red	—	29.3	28.5
Pelissier	57.8	61.3	62.7	4.0	3.3	2.6	26.0	29.7	31.7
Monad	60.8	62.7	64.3	5.0	3.3	3.0	27.0	29.8	30.9
Nodak	60.2	62.8	64.2	4.0	3.0	2.6	29.0	30.1	31.9
Kubanka	—	62.8	63.4	—	3.0	2.2	—	30.7	31.6
Acme	60.0	62.5	64.4	4.0	3.0	3.0	28.6	29.8	31.5
Pentad	—	63.2	64.5	Red	Red	Red	—	29.3	28.5
Golden Ball	57.0	61.2	61.4	5.0	4.3	3.6	24.8	29.7	29.8

[1] In computing the mean values, grades 1 C.W., 2 C.W., 3 C.W., 4 C.W., and 5 C.W. were assigned numerical values of 1, 2, 3, 4, and 5, respectively.

TABLE II

PROTEIN AND CAROTENE DATA [1]
Comparison of Mean Values—All Stations—Years 1935, 1936, and 1937

Variety	Protein content						Carotene content					
	Wheat			Semolina			Wheat			Semolina		
	1935	1936	1937	1935	1936	1937	1935	1936	1937	1935	1936	1937
	%	%	%	%	%	%	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*	*ppm.*
Arnautka	15.3	17.0	14.8	14.1	15.6	13.2	6.76	4.73	5.69	5.87	3.94	4.02
Mindum	14.3	17.3	14.1	13.2	15.7	12.8	5.79	4.56	6.23	6.39	3.62	4.60
Akrona	14.3	17.9	13.9	13.2	16.6	12.5	7.74	5.03	6.25	7.96	4.28	5.09
Iumillo	—	17.4	15.3	—	15.9	13.5	—	4.15	4.91	—	3.89	3.66
Pelissier	14.6	16.2	13.9	13.2	14.8	12.7	5.29	3.98	5.76	5.85	3.42	4.44
Monad	14.8	17.2	14.2	13.3	15.3	12.3	4.79	3.91	4.42	4.11	3.12	3.42
Nodak	14.3	16.3	14.0	12.8	14.7	12.3	5.63	4.09	5.01	5.35	3.48	3.82
Kubanka	—	19.6	14.1	—	14.7	12.5	—	4.18	5.13	—	2.22	3.94
Acme	13.8	16.6	14.3	12.6	14.5	12.5	4.62	3.57	4.39	4.19	2.99	3.21
Pentad	—	17.6	14.6	—	15.8	12.9	—	4.04	4.63	—	3.13	3.32
Golden Ball	16.7	16.7	14.8	14.4	14.2	12.6	5.62	5.18	6.86	5.39	3.81	5.08

[1] Results expressed on a 13.5% moisture basis.

TABLE III

BREAKING STRENGTH, TENDERNESS SCORE, AND COLOR SCORE OF MACARONI

Comparison of Mean Values—All Stations—Years 1935, 1936, and 1937

Variety	Breaking strength (arbitrary units)		Tenderness score (arbitrary units)			Computed color score		
	1936	1937	1935	1936	1937	1935	1936	1937
Arnautka	136	175	108.4	127.3	110.0	23.7	16.7	19.3
Mindum	158	177	99.3	98.1	113.4	24.4	17.7	18.4
Akrona	143	169	124.6	111.6	111.3	17.8	18.1	17.8
Iumillo	155	191	—	—	120.6	—	16.7	17.7
Pelissier	149	185	—	—	117.2	17.5	17.1	17.0
Monad	161	194	—	110.1	111.3	17.8	16.6	17.1
Nodak	157	183	—	—	117.0	16.8	16.7	16.9
Kubanka	163	185	—	—	119.1	—	15.6	16.7
Acme	168	198	—	—	113.1	16.0	15.4	16.5
Pentad	162	190	—	—	126.1	—	15.3	16.1
Golden Ball	138	179	—	—	116.6	15.7	16.0	15.8

TABLE IV

SUMMARY OF MEAN VALUES—ALL STATIONS—ALL YEARS [1]

Variety	Weight per Imperial bushel (cleaned wheat)	Official grade	Semolina yield	Protein [2]		Carotene [2]		Macaroni		
				Wheat	Semolina	Wheat	Semolina	Breaking strength	Tenderness score	Computed color score
	lbs.		%	%	%	*ppm.*	*ppm.*	*Arbitrary units*	*Arbitrary units*	
Arnautka	62.5	3.1	30.1	15.6	14.1	5.48	4.20	166	112.5	19.2
Mindum	63.2	2.7	31.5	15.2	13.8	5.62	4.49	170	107.0	19.0
Akrona	62.4	3.2	30.2	15.3	13.9	6.00	5.13	158	113.6	17.9
Iumillo	63.4	Red durum	28.8	16.2	14.3	4.63	3.62	176	120.2	17.3
Pelissier	61.7	3.3	30.3	14.7	13.6	5.10	3.99	169	117.2	17.1
Monad	63.3	3.8	30.1	15.2	13.4	4.29	3.40	180	111.0	17.0
Nodak	63.6	3.2	31.0	14.8	13.2	4.78	3.88	171	117.3	16.8
Kubanka	63.0	2.6	31.2	15.0	13.3	4.77	3.85	176	119.0	16.3
Acme	63.2	3.3	30.6	15.0	13.2	4.15	3.14	185	113.0	16.1
Pentad	64.0	Red durum	28.8	15.7	13.9	4.41	3.25	176	126.0	15.8
Golden Ball	60.8	4.2	28.0	15.6	13.3	6.16	4.70	162	116.5	15.8

[1] Varieties arranged in order of mean color scores.
[2] Results expressed on a 13.5% moisture basis.

TABLE V

COMPARISON OF INDIVIDUAL COLOR SCORE DATA

Variety	Morden			Brandon		Winnipeg		Melita	Indian Head
	1935	1936	1937	1936	1937	1936	1937	1937	1937
Arnautka	23.7	—	19.3	18.3	20.0	15.1	18.0	19.7	19.6
Mindum	24.4	16.8	19.4	18.6	19.4	—	16.8	17.1	19.3
Akrona	17.8	17.5	18.7	17.8	17.6	18.9	17.3	16.7	18.8
Iumillo	—	15.2	19.6	18.1	19.0	16.8	15.4	16.7	17.6
Pelissier	17.5	16.7	18.0	18.0	18.7	16.7	15.6	15.2	17.6
Monad	17.8	16.2	17.5	17.0	17.3	16.6	15.6	17.5	17.4
Nodak	16.8	16.5	19.2	18.0	18.2	15.6	15.3	14.6	17.4
Kubanka	—	15.5	18.2	16.1	18.2	15.1	15.7	14.3	17.2
Acme	16.0	16.2	17.6	16.3	18.0	13.8	14.5	15.6	16.6
Pentad	—	14.9	16.7	16.2	16.4	14.9	15.1	—	16.2
Golden Ball	15.7	15.8	17.5	16.4	16.4	15.7	15.3	12.5	17.3

each only three out of eight. An item of some interest is the relatively high placing accorded to Iumillo. On a visual basis, this variety would be placed close to the bottom of the list, because of the noticeable presence of bran specks in the macaroni. The red durums, Iumillo and Pentad, were included in the tests only to ascertain their value as parents in the production of high-quality rust-resistant hybrids, and it would appear that Iumillo possesses far better characteristics in this regard than Pentad. This conclusion has been borne out experimentally; of a number of rust-resistant hybrids examined in recent years, all the promising lines have originated from Iumillo-Mindum crosses.

The low placing of Kubanka is rather striking, as this variety is usually classed commercially along with Mindum as representing the two most satisfactory varieties. In the studies previously reported by Binnington and Geddes (1937), it was indicated that the sample of Kubanka employed was grown from a pure-line selection and was probably not typical of the variety as commercially grown. In the later studies reported here, representative commercial Kubanka seed was used and the results are accordingly free from this criticism. It is possible, as Fifield *et al.* (1937) point out, that this variety may be affected to a greater extent by unfavorable growing conditions than Mindum, but whatever the reason may be, it would appear to be a definitely undesirable variety for the durum-growing regions of western Canada.

Passing from the question of macaroni color to consideration of the other factors involved, it will be noted that for all stations and years weight per bushel is maintained at a fairly uniform level for most of the varieties. Test weight varies with environmental conditions

as shown by the mean values for each year and was at a generally low level in 1935, as a result of heavy stem-rust infection; within each year, however, Golden Ball and Pelissier show a definite tendency to low test weight. The yield of semolina is related to the weight per bushel, excepting in the case of the red durums. It must be emphasized that the experimental yields are low in comparison with those obtained in commercial mills, for the reason that the primary object is to secure a semolina essentially similar to the commercial product; with the short experimental milling system, this can only be accomplished at the expense of yield.

Protein content of the wheat is also greatly influenced by environmental factors but would appear also to be influenced to some extent by variety, Pelissier and Nodak tending to exhibit the lowest values and Golden Ball the highest. Wheat carotene content, however, is definitely a varietal characteristic, being lowest in Acme and highest in Golden Ball. Neither of these factors individually, however, is closely associated with macaroni color score.

The remaining macaroni-quality factors, transverse breaking strength and tenderness score, show essentially similar mean values regardless of variety, although Mindum appears to produce macaroni falling below the general level of tenderness.

A detailed examination of the results secured in the past four years suggested the possibility of some associations existing between certain of the properties studied, and the data were therefore submitted to statistical analysis; the results obtained are worthy of brief discussion.

Inter-varietal Relations between Wheat Protein, Semolina Protein, and Tenderness of Macaroni

Intra-station, inter-varietal correlations between wheat protein, semolina protein, and macaroni tenderness were computed for the 1937 crop data for four of the stations represented. From the results recorded in Table VI it will be noted that while there is a high correlation be-

TABLE VI

INTER-VARIETAL CORRELATIONS BETWEEN WHEAT PROTEIN, SEMOLINA PROTEIN, AND MACARONI TENDERNESS

Correlation between	Correlation coefficients [1]			
	Winnipeg	Brandon	Melita	Indian Head
Wheat protein and semolina protein	.900	.863	.977	.965
Wheat protein and tenderness score	−.015	.317	.423	.205
Semolina protein and tenderness score	−.016	.233	.337	.237

[1] Value of r at 5% pt. = .497.

tween wheat and semolina protein, the correlation between protein content and macaroni tenderness is not significant. These results suggest the possibility of marked inter-varietal variations in protein "quality."

Inter-varietal Relations between Pigment Content of Wheat, Semolina, and Macaroni

Use of the new butyl-alcohol solvent has made possible estimation of the pigment content of ground macaroni, as this solvent releases appreciable amounts of pigment which could not formerly be extracted with naphtha-alcohol.

For this study the mean values, over all stations, for each of the varieties from the 1937 crop were employed, and the results are recorded in Table VII.

TABLE VII

INTER-VARIETAL RELATIONS BETWEEN PIGMENT CONTENT OF WHEAT, SEMOLINA, AND MACARONI

Correlation between	Correlation coefficients [1]
Wheat carotene and semolina carotene	.426
Wheat carotene and macaroni carotene	.504
Semolina carotene and macaroni carotene	.585

[1] Value of r at 5% pt. = .217.

These correlations are lower than would be anticipated. Variations in the germ content of experimentally processed semolina might readily account for the relatively low relations between wheat and semolina carotene. While the use of n-butyl alcohol permits the recovery of larger quantities of pigment from macaroni than naphtha-alcohol, the values obtained with it rarely approach the corresponding semolina values, and variations in the residual unextracted carotene would influence the magnitude of the correlations between wheat and semolina carotene and macaroni carotene.

Relations between Wheat and Semolina Protein, and Carotene and Macaroni Color Scores

In view of the magnitudes of the correlations cited above, and also the possible effects of protein content upon macaroni color through its relation to translucency and vitreousness, the series of correlations recorded in Table VIII were computed for the available data from the 1935 and 1936 crops, no attempt being made to classify the results

according to variety, grade, location, or crop year. The correlations found are given in Table VIII.

TABLE VIII

RELATIONS BETWEEN WHEAT AND SEMOLINA PROTEIN, AND CAROTENE AND MACARONI COLOR SCORES

Correlation between	Correlation coefficients [1]
Wheat protein and wheat carotene	−.566
Wheat carotene and macaroni color score	.056
Wheat protein and macaroni color score	.025
Semolina protein and semolina carotene	−.562
Semolina protein and macaroni color score	.100
Semolina carotene and macaroni color score	.205

[1] Value of r at 5% pt. = .224.

These results suggest that no intra- or inter-varietal relations exist between the various properties with the exception of the negative correlations between protein and carotene.

In addition to the computations detailed above, a similar series of correlations was calculated for the 1936 crop data. Significant correlations were obtained only between: wheat and semolina protein, $r = .871$; wheat and semolina carotene, $r = .870$; and semolina carotene and *semolina* color score, $r = .805$. It should be pointed out that no inter-varietal relation exists between semolina color score and macaroni color score, and accordingly the correlation of 0.805 between semolina carotene and semolina color is of no utility in predicting macaroni color.

The principal result of the above statistical studies is to emphasize the fact that no single analytical factor can be relied upon for the inter-varietal prediction of macaroni quality, and the best index as yet available is the color of the finished product. It should be noted particularly that wheat carotene alone is valueless for the inter-varietal prediction of macaroni color.

Summary

Ninety-nine samples of durum wheat, representing 11 standard varieties produced in western Canada during the years 1935, 1936, and 1937, have been milled into semolina, and macaroni has been produced therefrom. Mean values are presented for each year, covering the following analytical factors: official grades, weight per bushel, semolina yield, wheat and semolina protein and carotene, macaroni breaking strength, and tenderness and color scores.

Of the varieties studied, only Arnautka, Mindum, and Akrona have

been found to produce macaroni of satisfactory commercial quality with a reasonable degree of consistency over the three-year period.

Weight per bushel and semolina yield are governed chiefly by environmental factors, whereas protein and carotene are to a greater extent influenced by variety.

Significant positive inter-varietal correlations were obtained only between wheat protein and semolina protein, wheat carotene and macaroni carotene, and semolina carotene and macaroni carotene. Negative inter-varietal correlations were found between wheat protein and wheat carotene and between semolina protein and semolina carotene.

It is emphasized that macaroni quality cannot be predicted from any single analytical test as yet applied to the wheat; in particular, for inter-varietal predictions, wheat carotene alone is valueless as an index of macaroni color.

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THE ANALYTICAL ERROR OF THE KJELDAHL NITROGEN TEST [1]

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The evaluation of analytical errors influencing the reliability of such important routine determinations of the cereal laboratory as moisture, protein, and ash has received considerable attention by the American Association of Cereal Chemists. For several years, the Committee on Methods of Analysis sponsored collaborative studies on these determinations, the results of which were subjected to statistical treatment by Treloar (1928, 1929, 1930, 1932, 1933). These studies clearly showed the existence of systematic errors between laboratories in addition to random errors of appreciable magnitude within laboratories. Assuming normality of the error distributions, error ranges that may be anticipated in extensive replication within laboratories were computed from the standard deviations of the replicate errors and practical permissible limits of accuracy set up.

Treloar (1932) has pointed out that one would expect a positive correlation between the magnitudes of the random error and of the constituent being determined, and that if such a correlation exists, standards of random error based on analyses of samples of widely varying protein content may be too lenient for some samples and too stringent for others. The A.A.C.C. collaborative studies were necessarily confined to a limited number of samples and it was thus impossible to test this hypothesis.

In our laboratory records, duplicate protein tests are available for several thousand samples of wheat covering a wide range in protein content and representing all grades of Western Canadian hard red spring wheat. A similar series of flours, but smaller in number, has also been tested. It seemed of interest to use these data for a further statistical study of the analytical errors of the Kjeldahl nitrogen test.

The Kjeldahl nitrogen test is conducted in this laboratory essentially as outlined in Cereal Laboratory Methods (American Association of Cereal Chemists, 1935) with certain modifications for convenience and speed. Both mercuric oxide and copper are employed as catalysts, sodium thiosulphate is used as a mercury precipitant, and 0.06265N H_2SO_4 and NaOH are used, the former being adjusted to correct for

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the blank. Inversely calibrated burettes are employed for back-titration so that the reading divided by 2 gives the percentage of protein directly. The tests are run in batches of 24; the digestion heating units are all standardized to a capacity of 570 watts and the distillation heating units to 800 watts; 40 to 45 minutes are allowed for digestion, while the distillation requires 20 to 25 minutes. Wheat samples (approximately 60 g.) are ground to a flour-like consistency in a Hobart burr mill, placed in ointment tins, and duplicate one-gram portions weighed from the same grind.

Results

The protein data available for statistical study comprised duplicate tests on 10,988 samples of wheat and 1,482 samples of flour; the range in protein content for wheat was 7.0% to 21.0% and for flour 7.0% to 18.0%. In classifying the data, a frequency table was compiled in which the differences between duplicates in 0.03% intervals were tabulated for each 1% increment in mean protein content.

Casual inspection of the frequency distributions given in Tables I and II reveals that, contrary to expectation, there is no relation between protein content and the magnitude of the error; the correlation coefficient was computed for the wheat errors and found to be $r = 0.056$ (5% point $= 0.019$). In view of this low correlation, the entire series of data can be combined in order to study the errors in protein determinations at all levels; these are graphically represented in Figures 1 and 2 for wheat and flour respectively.

Curve type was measured by application of R. A. Fisher's "k" statistics as outlined by Goulden (1936), in which two statistics, g_1 and g_2, are computed; g_1 is a measure of symmetry; a symmetrical curves gives a g value of zero while positive and negative values indicate positive and negative skewness. A positive value of g_2 indicates a peaked or leptokurtic curve, and a negative value a flat-topped or platykurtic curve. As would be expected, $g_1 = 0$, since theoretically one-half the errors would be considered positive and the other half negative; the g_2 values, however, for both wheat and flour were positive and highly significant and, hence, the error distributions are leptokurtic or peaked. In such curves, the center is higher and more pointed than normal and the tails are extended.

The explanation of leptokurtic distributions under routine conditions appears to lie in the occurrence of occasional gross discrepancies attributable to unnoticed accidents rather than ordinary experimental errors in an otherwise approximately normal system. In this connection, it is of interest to mention the results of an unpublished statistical study

TABLE I

Distribution of Wheat-Protein Analytical Errors Classified According to Protein Content

Difference between duplicates	Protein Content															Total	
	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.9	14.0–14.9	15.0–15.9	16.0–16.9	17.0–17.9	18.0–18.9	19.0–19.9	20.0–20.9	21.0–21.9		
% %																*No.*	%
.00–.02	4	9	18	28	114	236	268	414	248	90	69	25	19	9	1	1,552	14.11
.03–.05	2	9	25	45	128	242	279	474	253	104	45	10	4	1	1	1,622	14.76
.06–.08	—	6	22	29	79	199	214	324	211	81	20	6	1	2	—	1,194	10.87
.09–.11	—	8	15	37	123	251	300	542	267	114	36	10	7	7	1	1,718	15.64
.12–.14	1	2	10	15	60	138	161	256	157	72	21	5	1	1	—	900	8.19
.15–.17	1	3	7	18	66	155	165	337	199	71	17	10	—	—	—	1,049	9.55
.18–.20	1	2	6	12	71	135	181	323	159	77	40	22	25	23	2	1,079	9.82
.21–.23	—	—	2	9	23	81	120	203	133	33	9	—	2	—	—	615	5.60
.24–.26	—	—	3	7	11	42	64	86	62	34	9	2	—	—	—	320	2.91
.27–.29	—	1	—	2	1	6	26	41	39	13	7	1	—	—	—	137	1.25
.30–.32	—	1	—	2	2	45	25	40	45	16	9	3	—	1	1	190	1.73
.33–.35	—	—	—	3	2	5	13	19	20	6	4	1	—	—	—	73	0.66
.36–.38	—	—	—	—	3	6	15	15	15	6	4	1	—	2	—	67	0.61
.39–.41	—	—	—	1	4	3	11	18	14	7	6	2	—	—	—	66	0.60
.42–.44	—	—	—	—	2	2	4	6	7	3	1	—	—	—	—	25	0.23
.45–.47	—	1	—	1	—	6	10	14	14	4	2	—	—	—	1	53	0.48
.48–.50	—	—	1	1	—	3	4	4	10	9	2	2	1	1	—	38	0.35
.51–.53	—	—	—	—	—	1	3	4	7	1	—	—	—	1	—	17	0.15
.54–.56	—	—	—	—	—	3	2	5	7	2	1	—	—	—	—	20	0.18
.57–.59	—	—	—	1	1	1	1	4	3	—	—	—	1	—	—	12	0.11
.60–.62	—	—	—	—	2	2	4	2	1	1	2	1	—	—	—	15	0.14
.63–.65	—	—	—	—	—	2	1	1	3	—	2	1	—	—	—	10	0.09
.66–.68	—	—	—	—	—	1	1	—	1	1	—	1	—	—	—	5	0.05
.69–.71	—	—	—	—	1	1	5	8	2	1	1	1	—	1	—	21	0.19
.72–.74	—	—	—	—	—	2	2	3	1	1	1	—	—	—	—	10	0.09
.75–.77	—	1	—	—	—	2	1	3	4	1	—	—	—	—	—	12	0.11
.78–.80	—	1	2	1	8	10	10	8	7	5	3	2	1	—	—	58	0.53
.81–.83	—	—	1	—	2	3	2	6	1	2	—	—	—	—	—	17	0.15
.84–.86	—	—	—	—	1	6	13	10	5	—	—	—	—	—	—	35	0.32
.87–.89	—	1	3	—	2	—	—	4	3	1	—	—	—	—	—	14	0.13
.90–.92	—	1	—	2	1	—	6	10	8	3	—	—	—	—	—	31	0.28
.93–.95	—	—	1	3	1	—	1	1	1	—	1	—	—	—	—	9	0.08
.96–.98	—	—	—	—	—	—	1	1	—	2	—	—	—	—	—	4	0.04
Total:																	
Number	9	46	116	217	708	1,589	1,913	3,186	1,907	761	312	106	62	49	7	10,988	
Per cent	0.08	0.42	1.06	1.97	6.44	14.46	17.41	29.00	17.36	6.93	2.84	0.96	0.56	0.45	0.06		100.00

TABLE II

DISTRIBUTION OF FLOUR-PROTEIN ANALYTICAL ERRORS CLASSIFIED ACCORDING TO PROTEIN CONTENT

Difference between duplicates	Protein Content												Total	
	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.9	14.0–14.9	15.0–15.9	16.0–16.9	17.0–17.9	18.0–18.9		
% %													*No.*	%
.00–.02	3	5	4	8	20	43	76	39	42	19	2	10	271	18.29
.03–.05	1	4	8	13	26	54	61	47	51	11	1	1	278	18.76
.06–.08	4	4	2	6	16	21	34	27	27	8	—	—	149	10.05
.09–.11	1	4	3	8	19	42	52	56	32	6	—	5	228	15.38
.12–.14	1	2	1	7	7	23	27	25	20	1	—	—	114	7.69
.15–.17	—	1	2	4	19	23	31	29	18	5	—	1	133	8.97
.18–.20	—	—	1	4	14	28	38	25	19	10	2	5	146	9.85
.21–.23	—	1	2	2	4	10	14	15	13	2	—	—	63	4.25
.24–.26	—	—	—	—	2	6	6	6	9	4	—	—	33	2.23
.27–.29	—	—	1	—	2	1	6	5	2	2	—	—	19	1.28
.30–.32	—	—	—	—	1	2	2	1	2	—	—	—	8	0.54
.33–.35	—	—	—	—	1	—	2	2	2	—	—	—	7	0.47
.36–.38	—	—	—	—	—	1	1	—	6	—	1	—	9	0.61
.39–.41	—	—	—	1	—	—	3	3	2	—	—	—	9	0.61
.42–.44	—	—	—	—	—	—	1	—	1	—	—	—	2	0.13
.45–.47	—	—	—	—	—	—	3	—	—	—	—	—	3	0.20
.48–.50	—	—	—	—	—	2	1	—	—	1	—	—	4	0.27
.51–.53	—	—	—	—	—	—	1	—	—	—	—	—	1	0.07
.54–.56	—	—	—	—	—	—	—	—	—	1	—	—	1	0.07
.57–.59	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.60–.62	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.63–.65	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.66–.68	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.69–.71	—	—	—	—	—	—	—	—	—	1	—	—	1	0.07
.72–.74	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.75–.77	—	—	—	—	—	—	1	—	—	—	—	—	1	0.07
.78–.80	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.81–.83	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.84–.86	—	—	—	—	—	—	—	—	—	1	—	—	1	0.07
.87–.89	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.90–.92	—	—	—	—	—	—	1	—	—	—	—	—	1	0.07
Total:														
Number	10	21	24	53	131	256	361	280	246	72	6	22	1,482	
Percent	0.67	1.42	1.62	3.58	8.84	17.27	24.37	18.89	16.60	4.86	0.40	1.48		100.00

by J. W. Hopkins, Division of Biology and Agriculture, National Research Council of Canada, of a series of duplicate loaf volumes obtained in several Canadian cereal chemical laboratories co-operating in the work of the Associate Committee on Grain Research; the loaf volume deviations in all laboratories and by all formulas showed a definite leptokurtic tendency. In his confidential report to the co-operating laboratories, Hopkins pointed out that with such distributions the rejection of outlying observations may be expected to result in a normal distribution of enhanced precision and thus to improve the accuracy of the mean.

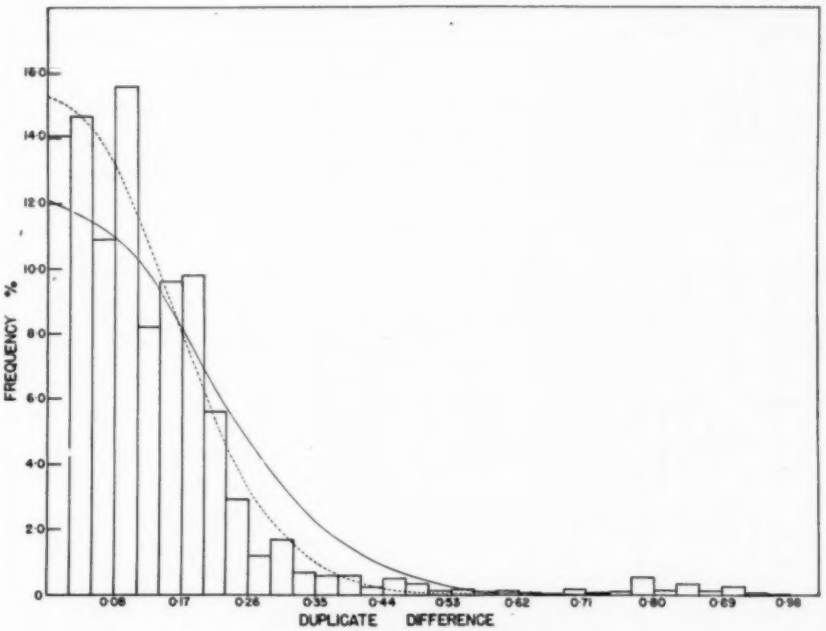

Fig. 1. Percentage frequency distribution of differences between duplicates for protein content of 10,988 wheats ranging from 7% to 21% in protein content. Histogram represents the actual errors, and the full-line continuous curve the fitted normal expectation. The broken line is the fitted curve for the errors from 0.00% to 0.50% inclusive.

On the other hand, when the error distribution is normal, there is no theoretical justification for the rejection of observations on the ground of their divergence. Increased accuracy in this case must be sought solely through additional replication or improvement in experimental technique.

The above considerations naturally suggest a means of arriving at the true random error and thereby of establishing reasonable limits within which duplicates should be expected to agree. The outlying classes may be successively discarded until a normal distribution results.

The upper error limit, when this condition is attained, will then represent the expected maximum error resulting from chance variations and the point above which one is justified in discarding a result in favour of an additional single determination which agrees with one or other of the previous duplicate tests within the prescribed range.

This technique was followed and, as the extreme error ranges were successively discarded, the positive values of g_2 decreased in magnitude. The wheat protein error curve was only slightly but significantly lepto-

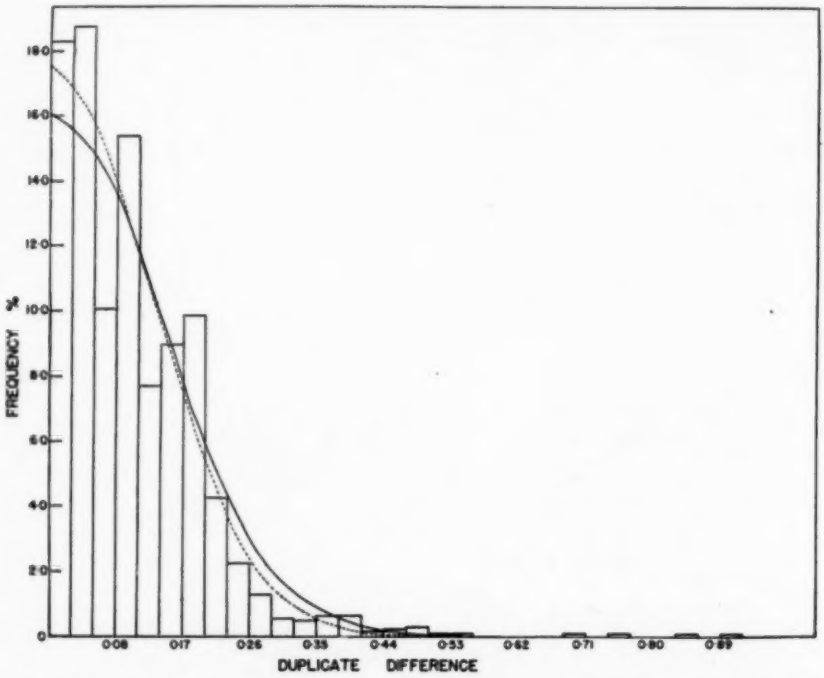

Fig. 2. Percentage frequency distribution of differences between duplicates for protein content of 1,482 flours ranging from 7% to 18% in protein content. Histogram represents the actual errors, and the full-line continuous curve the fitted normal expectation. The broken line is the fitted curve for the errors from 0.00% to 0.44% inclusive.

kurtic after all duplicate errors in excess of 0.50 were discarded; when the error class 0.48 to 0.50 was eliminated, a small but significant negative value for g_2 resulted. Owing to the large number of variates involved, the test of significance is very precise and for practical purposes it may be assumed that duplicate errors within the range of 0.00 to 0.50 may be considered as ordinary experimental or random errors, whereas discrepancies in excess of 0.50 are due to accidental systematic factors. Of the 10,988 samples included in this study, 290 or 2.64% fell into this latter category.

The flour-protein-error curve for the ranges 0.00 to 0.44 inclusive was "normal"; the elimination of the class 0.42% to 0.44% rendered the curve significantly platykurtic, whereas the inclusion of the class 0.45% to 0.47% resulted in a leptokurtic distribution. Accordingly, duplicate errors up to and including 0.44% for flour protein determinations in this laboratory may be considered random and there is no justification for rejecting such observations. However, if the error exceeds this, a single additional test may be conducted and the two most closely agreeing results of the three accepted as a more reliable measure of the mean than the average of the three, provided the differences between any two of the determinations fall within the required limits. Of the 1,482 samples, 13 or 0.9% possessed duplicate errors beyond the indicated range for random errors.

The means, ranges, and standard deviations for the duplicate errors of wheat and flour for both the leptokurtic and "normal" distributions are recorded in Table III. Considering only those errors which conform

TABLE III

STATISTICAL CONSTANTS FOR ANALYTICAL ERRORS OF THE PROTEIN DETERMINATION

	No. of samples	Protein content		Differences between duplicates		Standard error (single determination)	Curve type
		Mean	Range (approx.)	Mean	Range		
		%	%	%	%	%	
Wheat	10,988	14.18	7.0–21.0	0.140	0.00–0.98	0.139	Leptokurtic
Wheat	10,698	—	7.0–21.0	0.124	0.00–0.50	0.108	Slightly leptokurtic
Flour	1,482	13.63	7.0–18.0	0.112	0.00–0.92	0.103	Leptokurtic
Flour	1,469	—	7.0–18.0	0.108	0.00–0.44	0.096	Normal

to an approximately normal distribution, the mean differences between duplicates are 0.124% for wheat and 0.108% for flour; the corresponding standard errors (single determination) are 0.108% and 0.096% respectively. It is of interest to note that Whiteside (1936), in a study of duplicate protein tests on 336 wheat samples, found the standard error of a single determination to be 0.148%. In his study, the duplicate tests were also made on the same grind and hence this figure compares with that of 0.139% found in the present study for the standard error of the entire series of wheat samples. The differences between these statistics for wheat and flour have been tested and found to be highly significant. It is of interest to note that Treloar (1930) also found the protein errors for flour to be lower than for wheat. As previously pointed out by Treloar (1929), these errors are of such magni-

tude as to render the reporting of protein results to more than one decimal place unjustifiable.

The standard errors given in Table III may be utilized to compute the number of replicates required to secure any desired degree of accuracy merely by setting up the fiducial limits at the percent point required. At the 5% point the fiducial limits will be $\pm 1.96\ s/\sqrt{n}$ where s is the standard error of a single determination and n is the number of replicates. This is based on a t value of 1.96 on the grounds that the number of samples from which s has been calculated is quite large. The results of these calculations for the flour data are given in Table IV. If only a single protein test is run, the result may

TABLE IV

NUMBER OF REPLICATES REQUIRED FOR DIFFERENT LEVELS OF ACCURACY IN THE PROTEIN TEST ON FLOUR

Desired accuracy (Fiducial limits, plus or minus)	Number of replicates [1] (s = .096)
%	
0.20	1
0.15	2
0.10	4
0.05	14

[1] Given to nearest integer.

be expected to be within $\pm 0.2\%$ of the correct value; in order to secure an accuracy of 0.1%, it would be necessary to run four replicates.

The corresponding data for wheat have not been given, because the sampling error on whole wheat was not taken into account; these might suggest that the protein content of wheat can be determined with greater accuracy than is probably the case.

Discussion

This study, based as it is on such a large number of determinations, gives a very reliable measure of the analytical error of the Kjeldahl nitrogen test as carried out in this laboratory, and provides a practical approach for establishing standards of accuracy. The tests were made in the course of ordinary routine work and no special precautions were taken by the analysts such as is likely, consciously or unconsciously, to be the case when special studies are conducted for the purpose of evaluating errors.

It must be emphasized that the duplicate tests with wheat were made on the same grind and, hence, any error due to subsampling the grain

is not included in the statistical estimates of precision. That the error involved in sampling unground grain may frequently be a major source of error has been demonstrated by Cook, Hopkins, and Geddes (1934) in the instance of moisture determinations on wheat, and by Sallans and Anderson (1937) with respect to Lintner value determinations on malt. In view of these findings, it is altogether likely that the accuracy of the protein test, indicated in the present study, is greater than that which can be attained if the error in sampling the unground grain is taken into account. It is for this reason that the term "analytical error" rather than experimental error has been employed, since the latter properly includes errors due to sampling as well as those involved in the actual Kjeldahl determination. All samples of grain analyzed in laboratories represent some large bulk, such as a carload or the yield from a field plot, and these must be sampled at some stage of the determination. If this procedure is a serious source of error, the standard error of the protein test, if it is to be used as a criterion for determining whether two samples differ significantly, should be calculated from determinations made on duplicate subsamples ground separately. In these circumstances it appears that the principal object of making duplicate determinations on the same grind is to guard against gross errors resulting from unobserved accidents which are outside the range of normal analytical error.

It should be pointed out, however, that in many investigations, the laboratory error of the protein test, estimated from results of determinations made on duplicate samples ground separately, is not the proper one to use as a criterion of significance. A more suitable one such as a variance due to a differential effect is frequently available. This point is well illustrated in Whiteside's (1936) statistical study of protein data obtained on a series of 28 wheat varieties grown in quadruplicate rod-row plots at each of three stations. Not only was there a significant difference between the replicate plots of each variety at each station, but there was also a significant interaction between varieties and stations, showing that the protein contents of the different varieties did not bear the same relation to each other at all three stations. Within each station, the sampling or plot error exceeded the laboratory error and, in order to secure a valid basis of comparing the varieties, it was necessary to take account of the variability in the protein content of the wheat from the different plots. Accordingly, for any one station the error variance remaining after removal of the varietal effect and that due to replications from the total variance, was the most satisfactory one to employ as a measure of the significance of differences between varieties. When, however, variety tests are conducted at a number of locations, Whiteside (1936) has shown that the interaction variance for varieties

and stations rather than the error variance should be utilized to ascertain the significance of differences between the varietal means for all stations, since the former was found to be the larger.

The problem of obtaining a good estimate of the protein content of flour is simpler than in the instance of wheat. There is no sampling error corresponding to that for unground wheat, and because flour is a very finely ground, thoroughly mixed, and relatively homogeneous material, the sampling error resulting from taking fractions for analysis should be smaller than the corresponding sampling error for ground wheat. The results of the present investigation offer some support for this hypothesis since, if the sampling error for flour is not smaller than that for ground wheat, it is difficult to understand why the standard error for the determination of flour protein is significantly lower than that for wheat protein.

It is of interest to note that Treloar (1932), in a study of the protein errors of quadruplicate determinations, reported by 99 laboratories on a single flour sample, found that while the composite error curve was symmetrical, the errors in the extreme ranges appeared to be of greater frequency than the " normal " distribution would allow for. However, the distribution of error for individual laboratories may be normal but if the standard deviations of these errors be differentiated the composite curve of the errors of all collaborators combined would be leptokurtic. A study of the distribution of the standard deviations of error for the various laboratories indicated that such differentiation existed and could easily be responsible for the leptokurtic character of the composite error curve. Treloar (1932) points out that these observations cannot be interpreted as implying that the error distribution within laboratories is normal; he considers that the hypothesis of normality of error distribution provides a satisfactory basis of deduction for practical purposes, since any error it introduces would be small.

The data presented in this paper show quite conclusively that the protein-error curve is leptokurtic. Reference has been made to the fact that loaf-volume errors have also exhibited this type of abnormality. Upon reflection, it would be anticipated that in any laboratory where large numbers of routine determinations are being carried out daily, unobserved accidental discrepancies are bound to occur, which result in occasional errors of larger magnitude than the normal range. In general, then, leptokurtic distributions would seem to be the rule rather than the exception and this is a matter of great importance in establishing standards of error for such analytical procedures. With such distributions, the rejection of outlying observations is perfectly justified and may be expected to result in enhanced precision. A large number

of observations, however, are necessary in order to fix the range within which errors may be considered due to random causes.

Summary

A study of the analytical errors of duplicate protein tests on 10,988 wheats varying between 7% and 21% in protein content and on 1,482 samples of flour containing from 7% to 18% in protein reveals that no relation exists between protein content and the magnitude of the errors.

The analytical errors ranged from 0.00% to 0.98% for wheat and from 0.00% to 0.92% for flour; the corresponding mean errors were 0.140% and 0.112%, while the standard errors of a single determination were 0.139% and 0.103% respectively. As the duplicate tests with wheat were made on the one grind, any errors involved in sampling the whole grain are not included.

The error distributions were leptokurtic, indicating the occurrence of gross discrepancies, probably resulting from unnoticed minor accidents which are outside the range of normal analytical error. Successive rejection of outlying observations resulted in a normal error-distribution curve over the range of 0.00% to 0.50% for wheat and 0.00% to 0.44% for flour. These ranges represent the limits within which duplicate errors may deviate from random causes alone, and results which deviate beyond these limits may be discarded.

The random protein error for wheat is significantly higher than for flour; this is most probably the result of a greater sampling error due to the less homogeneous and less finely ground state of the former. The mean random errors are: for wheat 0.124% and for flour 0.108%; the corresponding standard errors of single determinations are 0.108% and 0.096% respectively. Assuming a negligible sampling error for flour, single tests may deviate from the true value by 0.2%, and in order to secure a protein result accurate to 0.1%, four replicates agreeing within the specified ranges for random error would be necessary.

Acknowledgments

The protein results employed in this study were accumulated under the supervision of W. J. Eva, Assistant Chemist, Grain Research Laboratory, Board of Grain Commissioners. The authors are indebted to C. H. Goulden, Dominion Rust Research Laboratory, for advice on the statistical treatment of the data, and to J. A. Anderson, National Research Council of Canada, for valuable suggestions on the interpretation of the data.

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A STUDY BY THE PAIRED FEEDING METHOD OF THE NUTRITIVE VALUE OF BREAD MADE WITH MILK SOLIDS[1]

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In 1937, this laboratory undertook the study of the comparative nutritive value of three types of dry bread crumb prepared from bread doughs containing 0%, 6% and 12% of milk solids based upon weight of flour. A preliminary report has been published (Fairbanks, 1938) in which the *ad libitum* method of feeding was employed and the conclusions drawn were based entirely upon differences in body weight. The plan of work was stated as follows: "It is the purpose of this present work to compare the over-all nutritive value of bread made without milk solids, with bread made with the addition of 6% milk solids (based upon weight of flour) and with bread made with the addition of 12% milk solids. The experimental technique employed in this initial work is not capable of explaining in terms of nutrition why observed differences occur. At present we are only interested in the differences, and further experiments which are better controlled and more refined in procedures will be conducted to explain that which has been observed and recorded here."

One must carefully avoid very emphatically expressed judgments based upon differences in body weights as obtained from *ad libitum*

[1] This investigation was made possible by the donation of funds to the University of Illinois by the American Dry Milk Institute, Inc.

feeding. Such differences may be due merely to differences in palatability rather than to differences in nutritive efficiency. The effects of palatability may be ruled out by modern methods of controlled feeding. Differences in body weights may also fail as an index to nutritive value, as they do not account for possible differences in body composition. This objection is readily overcome by making carcass analyses at the close of the feeding experiment.

This paper reports the results of an experiment in which the controlled-feeding technique was employed and the carcasses of the experimental animals were subjected to chemical analysis at the close of the feeding period. It is hoped that by this more critical experimental approach we may be able to confirm the conclusion previously reported (Fairbanks, 1938) that "The addition of milk solids to a water bread (no milk) formula increases the nutritive value of bread."

The bread samples used were the same as those studied in the preliminary experiment and, to conserve space, the reader is referred to the previous paper for a description of the samples, formula used, methods of baking, and preparation of the samples following baking. Bread samples were prepared at two different times and the chemical composition of both makes are presented in Table I. The chemical

TABLE I

CHEMICAL COMPOSITION OF THE EXPERIMENT NO-MILK-SOLIDS BREAD AND 12 PERCENT MILK-SOLIDS BREAD

Sample	Dry substance	Ether extract	Crude protein (N × 6.25)	Gross energy per g.	Ash	Calcium	Phosphorus
	%	%	%	*cals.*	%	%	%
No-milk bread, 1st make	96.08	0.98	15.88	4248	2.33	0.026	0.141
6% milk-solids bread, 1st make	96.27	0.86	17.01	4241	2.74	0.097	0.187
12% milk-solids bread, 1st make	96.41	0.90	17.68	4219	3.00	0.162	0.236
No-milk bread, 2nd make	95.22	0.95	13.08	4181	2.28	0.033	0.118
6% milk-solids bread, 2nd make	95.11	0.92	14.42	4160	2.63	0.108	0.180
12% milk-solids bread, 2nd make	95.66	0.85	15.76	4171	2.97	0.178	0.227

analyses indicate that the greatest differences occur in the protein, ash, calcium, and phosphorus contents of the no-milk, 6%, and 12% milk-solids bread.

The literature was reviewed and included in the former publication.

Plan of Experiment

The experimental technique employed was the paired-feeding method (Mitchell and Beadles, 1930) slightly modified to include three rats in each group rather than the customary two rats. Seven groups

of three albino rats each were carefully equated on the basis of litter, sex, and body weight. The first rat of each group was fed no-milk bread, the second rat received 6% milk-solids bread, and the third rat was fed 12% milk-solids bread. The sole diet of these experimental animals was bread and distilled water. This severe dietary regime coupled with the controlled method of feeding must be kept in mind as one studies the data.

The rats were confined in individual wire cages, and the food was weighed out daily to each individual rat. Food scattering was practically eliminated by mixing distilled water into the bread crumb after weighing. At the close of the experiment the rats were sacrificed, their body lengths taken immediately after death, and their carcasses saved for chemical analyses.

In the controlled feeding technique there are two methods that may be used. The first method prescribes the feeding of the rats of any one group of three rats so that the gains in body weight will be equal. This method rules out differences in maintenance requirements due to differences in body weights. The nutritive differences of the diets are indicated by differences in body lengths and food consumption for equal gains. The second method of controlled feeding is accomplished by feeding the same amount of feed to all rats of any one group. Differences in body lengths and differences in body weights may be regarded as measures of nutritive value. The carcass analyses contribute to the data obtained by either method of feeding.

When the experiment was initiated, it was planned to feed for equal gains. Because of the very poor performance of two groups of rats, it was deemed advisable at the end of 49 days to change over to the method of feeding equal amounts to the three rats of each group. When this change was made the rats were put under ether and their body lengths were taken. Thus for the first 49 days, data on food intake, body weights, and body lengths are available. After the change was made to equalized food intake, the experiment was continued for 56 days. Table II, which is divided into two parts, gives the essential data. The first part is for the 49 days on feeding for equal gains, the second part for the 56 days on equalized food intake.

If a rat of any one group died during the experiment, its group mates were sacrificed on the same day, and the three carcasses saved for chemical analysis. At the close of the experiment, three groups of rats had survived the rigorous experimental conditions and they were disposed of and their carcasses saved. Table III presents the chemical analysis of the seven groups consisting of 21 rats. All analyses are calculated on the dry-matter basis.

TABLE II

BODY LENGTHS AND BODY WEIGHTS, GAINS IN BODY WEIGHT AND FOOD CONSUMPTION ON THE THREE SAMPLES OF BREAD CRUMB
(All weights expressed in grams)

	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6			Group 7		
	No milk	6 %	12 %	No milk	6 %	12 %	No milk	6 %	12 %	No milk	6 %	12 %	No milk	6 %	12 %	No milk	6 %	12 %	No milk	6 %	12 %
Rat no. and sex	1f	2f	3f	4m	5m	6m	7f	8f	9f	10m	11m	12m	13f	14f	15f	16f	17f	18f	19m	20m	21m
FEEDING FOR EQUAL GAINS																					
Initial body weight	47	53	56	54	57	53	53	50	51	50	52	62	51	52	50	51	58	50	54	56	58
Final body weight	57	65	66	86	91	81	59	65	59	63	68	76	63	54	54	51	49	51	99	96	97
Gain	10	12	10	32	34	28	6	6	8	13	16	14	12	2	4	0	1	1	45	40	39
Body length, mm.[1]	144	155	158	163	174	187	151	153	153	153	157	167	154	163	163	148	149	158	168	172	177
Food	188	183	168	245	239	222	200	179	194	226	195	220	213	211	193	194	211	210	350	283	302
Length of test in days	49	49	49	49	49	49	49	49	49	49	49	49	44	44	44	49	49	49	49	49	49
EQUALIZED FOOD INTAKE																					
Initial body weight	—	—	—	86	91	81	59	56	59	63	68	76	—	—	—	51	49	51	99	96	97
Final body weight	—	—	—	98	119	118	75	85	88	68	86	84	—	—	—	53	53	60	77	97	104
Gain	—	—	—	12	28	37	16	29	29	5	18	8	—	—	—	2	4	9	−22	1	7
Body length, mm.[1]	—	—	—	165	176	189	157	166	167	160	172	177	—	—	—	152	157	161	168	182	188
Food	—	—	—	283	283	283	217	217	217	238	238	238	—	—	—	187	187	187	191	191	191
Length of test in days	—	—	—	49	49	49	56	56	56	56	56	56	—	—	—	56	56	56	32	32	32

[1] Measured from anus to tip of nose.

TABLE III

CHEMICAL COMPOSITION OF THE CARCASSES OF THE RATS RECEIVING NO-MILK BREAD, 6 PERCENT MILK-SOLIDS BREAD AND 12 PERCENT MILK-SOLIDS BREAD
(Results calculated on a dry-matter basis)

Bread	Rat no. and sex	Dry substance	Gross energy per gram	Protein (N × 6.25)	Ether extract	Ash	Calcium	Phosphorus
No-milk bread	1f	30.68	4955	67.03	14.64	9.71	2.47	1.93
6% milk bread	2f	29.43	5433	73.10	12.51	12.34	3.25	2.37
12% milk bread	3f	31.89	5115	75.77	8.35	13.60	3.56	2.60
No-milk bread	4m	34.10	5256	68.50	23.15	7.88	1.86	1.56
6% milk bread	5m	32.71	6362	64.44	34.85	9.70	2.54	1.77
12% milk bread	6m	34.89	5209	57.92	23.39	10.07	3.14	1.97
No-milk bread	7f	35.62	6833	60.95	35.04	8.91	2.29	1.71
6% milk bread	8f	34.49	5936	57.10	30.57	9.92	2.75	2.03
12% milk bread	9f	33.06	6405	56.81	33.79	11.72	3.50	2.26
No-milk bread	10m	32.37	5043	72.03	13.16	9.73	2.16	1.93
6% milk bread	11m	26.28	5787	78.83	17.26	13.91	4.03	2.72
12% milk bread	12m	30.18	4687	76.33	8.94	14.53	4.13	2.74
No-milk bread	13f	28.55	5212	79.84	10.01	11.48	2.96	2.24
6% milk bread	14f	35.04	3862	71.62	4.30	12.20	3.41	2.32
12% milk bread [1]	15f	—	—	—	—	—	—	—
No-milk bread	16f	34.36	5229	58.03	29.04	12.63	2.79	2.02
6% milk bread	17f	30.33	5828	67.02	18.68	14.97	4.50	2.85
12% milk bread	18f	35.10	4928	60.22	16.10	13.65	4.67	2.57
No-milk bread	19m	27.30	4429	88.54	4.58	11.16	2.90	2.13
6% milk bread	20m	32.50	5122	77.18	10.26	11.58	3.21	2.18
12% milk bread	21m	31.42	5063	79.69	11.61	13.13	3.67	2.68
Averages								
No-milk bread	—	—	5280	70.70	18.52	10.21	2.49	1.93
6% milk bread	—	—	5476	69.90	18.35	12.09	3.38	2.32
12% milk bread	—	—	5235	67.79	17.03	12.78	3.78	2.47

[1] Sample lost.

The data have been subjected to statistical analysis according to the method of Student (1908) and the statistical results are summarized in Table IV.

Results of the Experiment

Feeding for equal gains.—In the first part of Table II will be found the data for the 49 days of feeding for equal gains. It will be noted that we were not entirely successful in keeping the gains of all three rats in each group exactly the same. As the experiment progressed this became increasingly difficult, because of erratic appetites of the rats, especially those receiving the no-milk bread. For this reason, along with poor performance of Groups 5 and 6, the change in feeding was

made as has been previously mentioned. However, the data on body lengths and on food consumption may be used as indices of differences in the nutritive values of the three kinds of bread crumb.

The average body length of the rats receiving no-milk bread was 154 mm., for the rats fed 6% milk-solids bread, 160 mm., and for the rats on 12% milk-solids bread, 166 mm. If the differences obtained with the rats on the three kinds of bread are considered as paired observations, then Student's (1908) method for statistical analysis of small groups of such data may be applied. As seen in Table IV, the average difference in body length between the rats fed no-milk bread and 6% milk-solids bread is 6.00 mm., the standard deviation is 3.93, and the probability of a chance outcome only 0.0048. When the body lengths of the rats on no-milk bread are compared with those fed 12% milk-solids bread, the average difference is 11.71 mm., the standard deviation of excess length is 6.26, and the probability that fortuitous factors would have produced this outcome is only 0.0019. The figures for the differences in body length between 6% milk-solids bread and 12% milk-solids bread are 5.71 mm. for the difference, with a standard deviation of 4.72 and a probability figure of 0.0127. In all three instances the criterion of significance has been satisfied. Such statistical evidence supports the conclusion that the rats on the 6% and 12% milk-solids breads are significantly longer than the rats fed the no-milk bread, and that the rats on the 12% milk-solids bread are significantly longer than those fed 6% milk-solids bread. This observation is emphasized as there is a growing conviction in this laboratory that differences in body lengths are a more superior criterion of nutritive efficiency in the growing rat than differences in body weights.

The average food consumption of the rats receiving no-milk bread was 231 g., for those receiving 6% milk-solids bread it was 214 g., while the average consumption of the rats on 12% milk-solids bread was 216 g. As indicated in Table IV these differences in food consumption are not statistically significant. In other words significant differences in the body lengths of the three series of rats have been obtained on food intakes, the differences between which are not statistically significant, an observation that may be used as further evidence of the nutritional superiority of the breads containing the milk solids. In this part of the experiment the rats were being fed for equal gains, so greater nutritive efficiency would be indicated by a smaller food intake. It is admitted that a stronger case for the addition of milk solids could be presented if the food consumption of the rats receiving milk-solids bread decreased as the milk solids were increased, with observed differences that were statistically significant. Referring again to the statistical analyses in Table IV, it is interesting

TABLE IV

ANALYSIS ACCORDING TO STUDENT'S METHOD OF THE OBSERVED DIFFERENCES WITH RATS FED NO-MILK BREAD, 6 PERCENT MILK-SOLIDS BREAD AND 12 PERCENT MILK-SOLIDS BREAD

	Mean of difference m	Standard deviation of difference s	Probability p	Statistical significance [1]
Feeding for equal gains:				
Body lengths				
No milk *vs.* 6%	6.00	3.93	0.0048	S
No milk *vs.* 12%	11.71	6.26	0.0019	S
6% *vs.* 12%	5.71	4.72	0.0127	S
Food consumption				
No milk *vs.* 6%	16.43	24.94	0.0816	N
No milk *vs.* 12%	15.29	18.22	0.0432	N
6% *vs.* 12%	1.14	17.08	>0.4072	N
Equalized food intake:				
Gains				
No milk *vs.* 6%	13.40	6.77	0.0084	S
No milk *vs.* 12%	15.40	10.07	0.0189	S
6% *vs.* 12%	2.00	6.66	0.2909	N
Body lengths				
No milk *vs.* 6%	10.20	3.06	<0.0019	S
No milk *vs.* 12%	16.00	5.76	0.0026	S
6% *vs.* 12%	5.80	3.97	0.0218	S
Carcass analysis:				
Protein				
No milk *vs.* 6%	0.80	7.44	0.3985	N
No milk *vs.* 12%	1.39	7.02	0.3366	N
6% *vs.* 12%	1.82	3.84	0.1718	N
Ether extract				
No milk *vs.* 6%	0.17	7.07	0.4072	N
No milk *vs.* 12%	2.91	6.12	0.1670	N
6% *vs.* 12%	3.66	5.10	0.0846	N
Energy				
No milk *vs.* 6%	196.14	862.07	0.2977	N
No milk *vs.* 12%	56.33	367.71	0.3762	N
6% *vs.* 12%	510.17	593.34	0.0568	N
Ash				
No milk *vs.* 6%	1.88	1.22	0.0047	S
No milk *vs.* 12%	2.78	1.25	0.0021	S
6% *vs.* 12%	0.71	1.04	0.0950	N
Calcium				
No milk *vs.* 6%	0.89	0.59	0.0051	S
No milk *vs.* 12%	1.37	0.42	<0.0006	S
6% *vs.* 12%	0.40	0.23	0.0058	S
Phosphorus				
No milk *vs.* 6%	0.39	0.29	0.0085	S
No milk *vs.* 12%	0.59	0.12	<0.0006	S
6% *vs.* 12%	0.15	0.24	0.1098	N

[1] S = significant, N = not significant.

to note that this situation was approached in the comparison between no-milk and 6% milk-solids bread and again between no-milk bread and 12% milk-solids bread. While the differences are not statistically significant and the data are therefore not conclusive, the probability values do suggest that the 6% milk-solids bread and the 12% milk-solids bread when compared to the no-milk bread have produced equal gains on less food intake. This is particularly true in the comparison of no-milk bread with 6% milk-solids bread, as the probability figure is 0.0432. It must be remembered that the numbers of paired observations are small and the experimental conditions imposed are quite severe, making for erratic appetites.

The observations during the period of feeding for equal gains substantiates the conclusion that both the 6% and 12% milk-solids bread are more nutritious than the no-milk bread and that the 12% milk-solids bread has a higher nutritive value than the 6% milk-solids bread.

Equalized food intake.—The data during the period of feeding the same amount of food to the rats of any one group are presented in the second part of Table II. In this phase of the experiment, differences in nutritional efficiency are demonstrated by differences in gains in body weight and differences in body lengths.

The average gain in body weight for the rats fed no-milk bread was 2.6 g., on 6% milk-solids bread, 16 g., and on 12% milk-solids bread, 18 g. Further evidence of the improvement in nutritive value of bread by the addition of milk solids is afforded by the significant differences of the gains between no-milk bread and 6% milk-solids bread, as the probability of a chance outcome is 0.0084 (Table IV). The gains between the no-milk bread and the 12% milk-solids bread is likewise statistically significant with a probability of 0.0189. However, in this particular measurement we are unable to show a significant difference between gains in body weight of rats fed 6% and 12% milk-solids bread. As seen in Table IV the mean of the difference is only 2 g. in favor of the 12% milk-solids bread, while the standard deviation of the difference is 6.66 with a probability of 0.2909, which is definitely not significant.

At the close of the second phase of the experiment, body lengths were again measured and are included in Table II. The statistical analyses of these data are presented in Table IV. The rats receiving 6% milk-solids bread and 12% milk-solids bread were significantly longer than those receiving no-milk bread. Further, the rats fed 12% milk-solids bread were significantly longer than those on 6% milk-solids bread. It must be pointed out that while these differences in body lengths are due to differences in the nutritive values of the three

kinds of bread, the differences have accrued during both periods of the experiment. The increases in body lengths for the period of equalized food intake can be calculated from the data in Table II, but they do not contribute appreciably to the experiment since they are increases in body lengths as well as very significant differences previously reported for the period of feeding for equal gains. In other words the rats were not equal in body lengths at the beginning of the second period and as the supplemented-bread rats were significantly longer this puts them at a disadvantage when increases in body length are studied for the second period only.

Carcass analyses: Protein.—As shown by the chemical analyses in Table III and the summary of the statistical treatment of the data in Table IV, the addition of 6% of milk-solids or 12% of milk-solids to the bread formula had no effect upon the protein content of the rats. It must be admitted that it is very difficult to explain why the addition of milk solids to bread will cause a significant difference in the body lengths and not cause a like difference in the protein content of the carcasses. The biological values of the proteins of the three samples of bread should be determined by the nitrogen-balance method as developed in this laboratory (Mitchell, 1924a, and Mitchell and Carman, 1926). High biological values for the proteins of milk solids prepared by the spray process have been reported by Mitchell (1924b), Mitchell and Carman (1926), and by Fairbanks and Mitchell (1935). Mitchell and Smuts (1932) have demonstrated by the paired feeding method that wheat is deficient in the indispensable amino acid lysine, and that when lysine is added there is a large increase in the growth-promoting value of wheat. Mitchell and Carman (1926) report an average biological value of the protein of white flour of 52, and in a mixed diet containing white-flour protein and milk protein in the ratio of 2 to 1, the average biological value was 71. McCollum, Simmonds, and Parsons (1921) found milk to be an effective supplement to wheat with respect to protein, while Rose, MacLeod, and Bisbey (1923–4) working with human subjects report a protein storage in 12 days of 30.37 grams on bread and milk as compared to 41.44 grams on milk.

Ether extract and energy.—The data as presented in Tables III and IV indicate that the addition of milk solids to the bread formula has no significant effect upon the fat (ether extract) and total calories (energy) of the carcasses.

Ash, calcium, and phosphorus.—One of the interesting observations of this experiment is the improvement in the ash, calcium, and phosphorus contents of the rats by the addition of milk solids to bread. The analytical data are given in Table III and the statistical analyses of these data are included in Table IV. The rats on 6% and 12%

milk-solids bread have a significantly higher ash content than their pair mates receiving no-milk bread. The differences in the ash content of the rats on 6% milk-solids bread and 12% milk-solids bread are not statistically significant. Like results are observed for phosphorus. The differences in phosphorus between no-milk bread and 6% milk-solids bread and between no-milk bread and 12% milk-solids are significant, while these differences are insignificant between 6% and 12% milk-solids bread. But in the case of calcium all observed differences are statistically significant. The 6% and 12% milk-solids bread significantly increase the calcium content of the carcasses as compared to no-milk bread, and the rats on 12% milk-solids bread have a higher calcium content than their pair mates receiving 6% milk-solids bread.

Conclusions

By the method of feeding for equal gains, rats receiving milk-solids bread were significantly longer than those on no-milk bread, and these significant differences were the result of food intakes that were not significantly different in amounts. The rats receiving 12% milk-solids bread were significantly longer than those fed 6% milk-solids bread.

By the method of feeding equal amounts of food to paired individuals, it has been demonstrated that 6% and 12% milk-solids bread produced significantly larger gains and significantly longer rats than the no-milk bread. Between the 6% and 12% milk-solids bread groups, the differences in gains were not statistically significant, but the differences in body lengths were significantly different in favor of the 12% milk-solids bread.

The addition of milk solids to bread had no significant effect upon the protein, gross energy, or fat content of the rats.

The addition of 6% and 12% of milk solids to bread caused a significant increase in the ash and phosphorus content of the carcasses as compared to the no-milk bread, but these differences were not significant between the 6% and 12% milk-solids bread.

The addition of 6% and 12% of milk solids to bread caused a significant increase in the calcium content of the carcasses as compared to the no-milk bread, and, further, the calcium content of the rats fed 12% milk-solids bread was significantly higher than the calcium content of the rats receiving 6% milk-solids bread.

By the controlled method of feeding, the previous conclusion (Fairbanks, 1938) that the addition of milk solids to a no-milk bread formula increases the nutritive value of the bread has been confirmed.

The burden of the evidence supports the conclusion that in practical nutrition the nutritive value of the 12% milk-solids bread is of a higher order than that of the 6% milk-solids bread.

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STUDIES ON ALL-PURPOSE FLOUR

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Although much work has been done during recent years on the chemical properties and baking qualities of various flours, relatively little attention appears to have been paid to all-purpose flour, that widely selling variety most commonly used by housewives. This is in spite of the fact that the number of flours which bear the name "all-purpose" is legion. Flours from all over the country, and in a widely varying price range, are labeled in this way. When it is considered that these flours represent not only different varieties of wheat but also differences in consumer demand, it seems not unlikely that there might

be necessity for some elasticity in the use of the term. In view of these considerations a study of the baking qualities of several all-purpose flours from different wheat-growing and milling centers seemed desirable.

Five flours were chosen for the study. One was a Texas product, one was from Indiana, another was milled in Kansas City, while the remaining two were milled in Minnesota but were of different protein contents.

These flours were analyzed for total nitrogen and moisture content by the official methods of the Association of Official Agricultural Chemists. They were also used to make butter cake, yeast bread, and baking-powder biscuits, all products widely prepared by housewives. The products, which were baked by standard recipes under controlled conditions, were judged by both subjective and objective methods.

Table I shows the results of the chemical determinations and the averages of the judges' scores on three series of each product. The highest possible score in each case was 100.

TABLE I

CHEMICAL RESULTS AND JUDGES' SCORES FOR FIVE ALL-PURPOSE FLOURS

Flour	Protein content	Moisture content	Score on cakes	Score on bread	Score on biscuits	Total score
	%	%				
Texas brand	10.91	12.03	91.2	87.6	86.3	265
Indiana brand	10.54	12.71	87.5	86.2	85.9	259
Minnesota brand 1	10.98	12.43	90.1	87.9	83.3	260
Minnesota brand 2	11.70	13.01	90.7	89.3	88.6	269
Kansas City brand	10.89	11.85	90.2	85.4	88.1	263

It will be seen that the flour with the highest protein content was marked by the judges as making the best loaf of bread. This is as might be expected, since it has been shown that there tends to be a relationship between baking quality and protein quantity. Flours with a high protein content appear to be best for bread, while lower-protein flours make better cakes. In this study, however, the flour with the lowest protein percentage had the lowest rating on cakes.

The figures in the final column of Table I show that scores for all of the flours do not vary widely, indicating that products of a fairly similar nature were obtained. As a general rule, a flour which scored high on one product usually scored high on the others, too.

Several objective tests were tried on the baked products. For the cakes the index to volume and the compressibility were determined

according to methods described by Platt (1930) and Platt and Kratz (1933), and the moisture and sand-retention tests of Swartz (1938) were also carried out. In the experimental work with bread, the index to volume, compressibility, oven spring, and amount of water required to make a dough were determined. Biscuits were rated only on the basis of judges' scores. The various tests are described below.

By use of a planimeter, the surface area of the center section of each cake was determined. In making the measurements, the cakes were cut through the center, the exposed surfaces were placed against a piece of paper, and the outline traced in pencil. Two readings, checking within 0.02 square inch, were taken for each half. The results were averaged and reported as the surface area. There is a relation between surface area and volume, and it is possible to calculate the volume of the cake in cubic centimeters from this value. In this study, however, the method of Platt and Kratz (1933) was followed, and the planimeter readings themselves are reported as the "index to volume." This index for cakes made from each of the all-purpose flours varied only slightly. It might be observed, however, that the cakes which the judges scored highest had a slightly higher volume, while those with the lowest score also had the smallest volume.

The compressibility of cakes made from each flour was determined by use of the compressometer, a device developed by Platt (1930). By this method a weight is allowed to rest on the cut surface of the cake for one minute. It was observed that high compressibility was not necessarily an indication of quality for all-purpose flour cakes. Rather, it appeared that a cake having a coarse grain offered less resistance to the plunger of the compressometer than those with a fine grain and smooth texture.

The moisture absorption test is, to a certain extent, a measure of the eating qualities of a cake. Swartz (1938) suggests that probably the dry, unpleasant feel of a poor cake in the mouth is due to the fact that it does not absorb the saliva as rapidly as a more moist one. In performing the test, samples of cake are cut, weighed, placed in a petri-dish cover containing 30 cc. of water, and allowed to remain for exactly five seconds. At the end of that time the sample is removed, inverted, and quickly reweighed. The difference in grams between the two weights is taken as a measure of the absorptive power of the cake. The test may also be used as a measure of keeping qualities, since the cakes tend to absorb less moisture as they become drier and older. Little difference could be seen in moisture-absorption powers between the cakes made from the different flours, the average increase in weight being 11.30 grams.

The sand-retention test is designed as a relative measure of the size of grain of cakes. The test is made by cutting a sample of cake of definite size, weighing it, covering it liberally with sand of a known fineness, removing the excess sand by rotating the sample once at a 40-degree angle, and weighing again. A cake having a coarse, open grain should retain more sand than a fine-textured one. Here again no significant difference could be seen between cakes made from different all-purpose flours.

It should be stated that while cakes made from each of the all-purpose flours were good on the whole, none of them had as velvety a texture nor as even a grain as cake-flour cakes made under the same conditions. One series of cake-flour cakes was made during the course of the experiment. It was observed that while all-purpose-flour cakes have a smaller volume, are less compressible, and have a smaller moisture-absorption power than cakes made from cake flour, they appear to have better keeping qualities.

Table II shows the results of the work on cakes. The figures represent the averages of determinations on nine cakes from each all-purpose flour, and three cake-flour cakes.

TABLE II

RESULTS OF OBJECTIVE TESTS ON CAKES

Flour	Index to volume	Compressibility [1]			Moisture absorption [1]			Sand retention [1]
		1st day	2nd day	4th day	Fresh sample	After 3 days	After 1 wk.	
	sq.in.	*mm.*	*mm.*	*mm.*	*g.*	*g.*	*g.*	*g.*
Texas brand	9.75	24.8	17.7	13.1	11.83	11.18	7.98	1.61
Indiana brand	9.38	25.9	19.0	13.9	11.62	10.30	8.70	1.65
Minnesota brand 2	9.67	23.3	17.9	14.2	11.24	9.13	8.67	1.69
Minnesota brand 1	9.67	23.9	19.1	15.1	10.49	10.35	5.79	1.45
Kansas City brand	9.62	23.1	15.6	13.5	11.36	8.51	8.08	1.61
Cake flour	11.61	32.2	22.7	14.9	14.28	8.70	—	1.62

[1] Determinations made on samples 2 inches in diameter and 1 inch thick.

The index to volume of the breads was measured in the same way as that of the cakes. The volume of loaves made from Minnesota flour 2, that having the highest protein content, was found to be the largest, while those made from the Indiana flour, which had the smallest protein content, had the smallest volume.

When the compressibility of the breads was measured, four of the flours showed similar results, with an average compressibility on the first test of 11.9 mm. Bread made from Minnesota brand 1, however,

showed considerably greater compressibility than the other breads, its compressibility on the first test averaging 16.2 mm. The reason for this is not clear.

No significant difference could be seen between the flours in oven spring, or in the amount of water required to make a dough.

The results of the objective tests on bread are summarized in Table III.

TABLE III

RESULTS OF OBJECTIVE TESTS ON BREAD

Flour	Volume	Compressibility		
		1st day	2nd day	4th day
	sq.in.	*mm.*	*mm.*	*mm.*
Texas brand	14.07	11.2	7.9	6.1
Minnesota brand 2	14.56	12.3	8.1	6.2
Minnesota brand 1	14.18	16.2	11.1	8.9
Kansas City brand	13.60	13.3	9.3	6.6
Indiana brand	13.17	10.9	8.7	6.6

In the case of biscuits, it was found that on the main points of texture, flavor, crumb color, and volume, the flours ranked high as a whole. There was considerable variation on the points of symmetry, crust color, and character of crust.

It appears that although the five flours came from different parts of the country, had different protein contents and doubtless represented a number of varieties of wheat, the products made from them showed relatively little variation. It is to be remembered that flours may vary greatly in their baking qualities with changes in recipe or in methods of mixing. The standard conditions set up in this experiment were not necessarily the ideal ones for all of the flours. The results were similar enough so that it was felt that with careful though not necessarily similar handling, good products could be obtained from all of the flours. On the basis of these conclusions, therefore, it appears that all of the flours tested may rightfully bear the label "all-purpose."

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OBSERVATIONS ON THE HYDROGEN-ION CONCENTRATION OF CAKES [1]

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(Received for publication November 11, 1938)

The optimum hydrogen-ion concentration of various types of cakes for best flavor and appearance has not been definitely known. While the effects of increased acidity or alkalinity on the cake volume, grain, and texture can be determined quite accurately, the measurement of flavor and eating quality is difficult because of different individual preferences. Observations on the pH of white and yellow layer cakes and the effect of certain materials on the pH of some cakes will be presented here.

In one series of tests a yellow layer cake was baked after the following basic formula: 100% cake flour, 50% shortening (hydrogenated), 125% sugar (2X), 50% whole eggs (frozen), 3% salt, 7% baking powder (phosphate), 15% dry milk solids,[3] and 87% water and flavor. All mixing and baking procedures were maintained constant. The percentages of the various ingredients were varied and the resulting changes in pH of the cake were observed. The pH was obtained by shaking 10 g. of cake crumb in 50 c.c. of distilled water and allowing this to stand for 30 minutes with thorough shaking at intervals. The suspension was then centrifuged and the supernatant liquid was immediately used for pH determinations by means of a potentiometer. Garnatz (1937) showed that extracts of baked products will decrease in pH upon standing, so delays were avoided in the final stages of the determination.

The results of the tests indicated that variations within limits in the amount of shortening, sugar, whole eggs, and water did not affect the pH of the cakes appreciably. Baking powder was used at 6.5, 7.0, and 7.5% levels and the cakes had pH values of 7.35, 7.74, and 7.91, respectively. Thus a variation in the amount of baking powder in the batter produces a considerable change in pH of the cake. Dry milk solids used at levels of 8, 15, 20, 25, and 30% and with variations in the amount of water to maintain a constant viscosity of the batter resulted in pH values of 7.70, 7.41, 7.56, 7.31, and 7.29, respectively. This indicates a slight trend of the pH toward neutrality with increasing amounts of milk solids in the instance of this particular cake formula.

[1] Paper No. 1660, Journal Series, Minnesota Agricultural Experiment Station, St. Paul.
[2] American Dry Milk Institute Research Associate, University of Minnesota.
[3] Dry milk solids is the product resulting from the removal of fat and water from milk. It contains not over 1½% butterfat and not over 5% moisture.

Similar studies were also made on white layer cakes and the results were practically the same as those described for the yellow layer cakes.

One series of yellow and one of white layer-cake batters were then made, to which various increments of sodium bicarbonate or potassium acid tartrate were added in addition to the regular amount of phosphate baking powder. These cakes were scored for color of crust, symmetry, volume, texture, grain, crumb color, and eating quality. The same yellow-cake formula was used as before. The white-cake

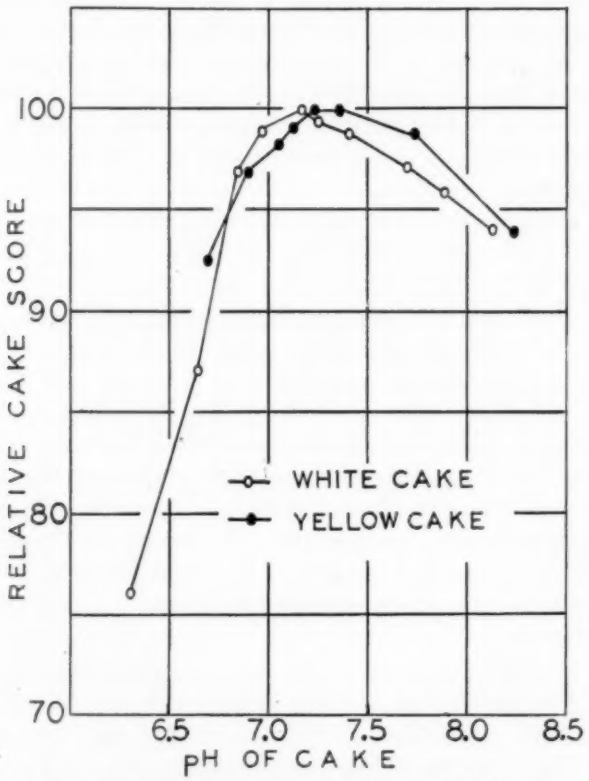

Fig. 1. Showing the effect of pH of the cakes on the final scores.

basic formula was as follows: 100% cake flour, 110% sugar, 52.5% shortening, 50% egg white (frozen), 30% dry milk solids, 7% baking powder, 3.75% salt, and 90% flavor and water. The flavoring substance used in all tests was a very small amount of pure vanilla extract. The scores obtained for the cakes on the basis of 100 for the best cake are shown in relation to the pH of the cakes by the graph in Figure 1. The highest scores were on the slightly alkaline side with both types of cakes and within a pH range from 7.00 to 7.90. The cakes with a pH

in this range were superior in all respects to the cakes which were either more acid or more alkaline.

White cakes at pH 8.15 had a dark crust and a yellowish crumb and this was also noticed to some extent at pH 7.88. The yellowish crumb color produced in white cakes by high alkalinity is probably due to a change in color of some of the flour pigments and also to greater caramelization of the sugars at the higher pH, particularly in the crust. When the acidity was increased beyond neutrality (pH 7.0) the crust color became appreciably lighter, in both the yellow and the white cakes.

To obtain some additional opinions relating to flavor, a group of ten persons were asked to judge the cakes as to preference. The yellow cakes at pH 7.19 and 7.25 were generally preferred, although a pH range of 6.98 to 7.88 was also acceptable to some persons. The acceptable white cakes were in the range from pH 7.05 to 7.89, but the cakes at pH 7.22 and 7.35 were commonly regarded as superior. Judges who were asked to taste cakes which were a day old did not as a rule exactly duplicate their opinions of the fresh cakes, but their selections were within the pH range of 7.10 to 7.80.

The series of tests just described were duplicated except that a commercial tartrate baking powder was used instead of the phosphate baking powder, with practically the same results. It was observed, however, that from the batters with no added soda or cream of tartar the tartrate-baking-powder cakes were at pH 7.13 and 6.90 and the phosphate-baking-powder cakes at pH 7.74 and 7.71 for the yellow and the white cakes, respectively. Thus the phosphate powder was balanced to give a more alkaline reaction than the tartrate powder that was used.

To study the effect of aging upon pH, white cakes made with tartrate baking powder were stored in a cabinet for three days at room temperature. The resulting data are shown in Table I. There was

TABLE I

EFFECT ON PH OF WHITE LAYER CAKES UPON THREE DAYS' STORAGE AT ROOM TEMPERATURE

Fresh cakes	8.06	7.96	7.67	7.50	7.15	6.90	6.83	6.78	6.67
Three days old	7.90	7.86	7.57	7.47	7.10	6.93	6.88	6.88	6.76

only a slight change in pH during the three days of storage. The small change appears to be toward neutrality of both the acid and the alkaline cakes. This is in agreement with the observations of Karacsonyi (1928) on the change in pH of bread stored at room temperature for 48 hours. He concluded that the acidity either remained constant or showed some decrease upon storage.

Several white, yellow, chocolate, and angel food cakes from various commercial bakeries were obtained and the pH was determined. The values are shown in Table II. The average pH for white cakes was

TABLE II

THE PH OF SOME COMMERCIAL CAKES FROM VARIOUS TYPES OF BAKERIES

Type of bakery	White cakes	Yellow cakes	Chocolate cakes	Angel food cakes
Chain retail	7.08	—	7.96	5.33
Chain retail	7.29	—	7.93	—
Chain retail	7.68	—	8.63	—
Retail	7.22	7.68	8.89	6.48
Retail	7.95	7.69	8.81	—
Retail	—	—	8.47	—
Wholesale	7.61	7.39	8.49	5.44
Wholesale	—	—	8.71	5.43
Av. pH	7.47	7.59	8.48	5.67

7.47, yellow cakes 7.59, chocolate cakes 8.48, and angel food cakes 5.67. Most of the commercial white and yellow cakes were within the optimum pH range as previously determined by this study.

Glabau (1938) placed the optimum pH for white and yellow cakes at about pH 7.00, but it should be noted that the pH differences between the individual cakes in his studies were rather large. Accordingly it became difficult to narrow down the optimum range within the smaller limits, which was attempted in this study. He pointed out that pH control of cakes is important and that cakes with egg yolks and no whites will have a higher acidity than cakes with whole eggs, and these in turn higher than with egg white when all other factors are constant.

Cake ingredients such as baking powder, eggs, flour, and liquid milk from different sources vary appreciably in their buffering action in the cake batter. Six lots of spray-process dry milk solids were available and yellow cakes baked with 30% milk solids had pH values of 7.12, 7.34, 7.27, 7.25, 7.41, and 7.27, respectively. In these instances all cakes scored high and were of good quality, and apparently these different high-grade milk solids varied only slightly in their effect on the pH of the finished cake.

Summary

Baking powder, in terms of both kind and quantity, affected the pH of white and yellow layer cakes more than any of the other common ingredients.

Optimum pH range of white and yellow layer cakes, in terms of preferred flavor and eating qualities, was 7.0–7.9. It appears that this

range could be narrowed down to 7.22–7.35. Cakes with a pH value outside the range of 7.0–7.9 were distinctly inferior when baked with either a phosphate or a tartrate baking powder.

Dry milk solids affected the pH of cake to a limited and probably an insignificant degree when used in proportions of 8 to 30 parts per 100 parts of flour in the cake formula. Likewise, there was only a small difference in the pH of cakes made with six different commercial lots of high-grade dry milk solids.

Storing cakes for three days did not result in an appreciable change in pH.

Average pH value of commercial white cakes was 7.47; of commercial yellow cakes 7.59; of chocolate cakes 8.48; and of angel food cakes 5.67.

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"PHOTO-RECORDS" AS APPLIED TO CAKE

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In a previous article a method and apparatus were described for making permanent records of baking studies.[1] Most of the work reported in this first article dealt with bread. One illustration of pound cake was shown and it was only fairly satisfactory. All pictures of cake obtained up to that time were more or less "fogged" around the edges because of stray light. This article presents some additional work in the production of "photo-records" of cakes.

Apparatus

All of the pictures recorded here were made with the "photo-record" apparatus[2] which was described briefly in the supplement of the pre-

[1] These projected photographic pictures will be referred to as "photo-records." The previous article is Wm. H. Cathcart, A Practical Method of Obtaining Permanent Records of Baking Studies, Cereal Chem. **15**: 775–787.

[2] Manufactured by the National Mfg. Company, Lincoln, Nebraska.

vious article. An anastigmat lens was used (cost approximately $25.00).

Four No. 1 photo-flood bulbs, placed close together in the same plane, were used as the light source. A ground-glass plate was used as before to make the light even and diffused. The section of the apparatus con-

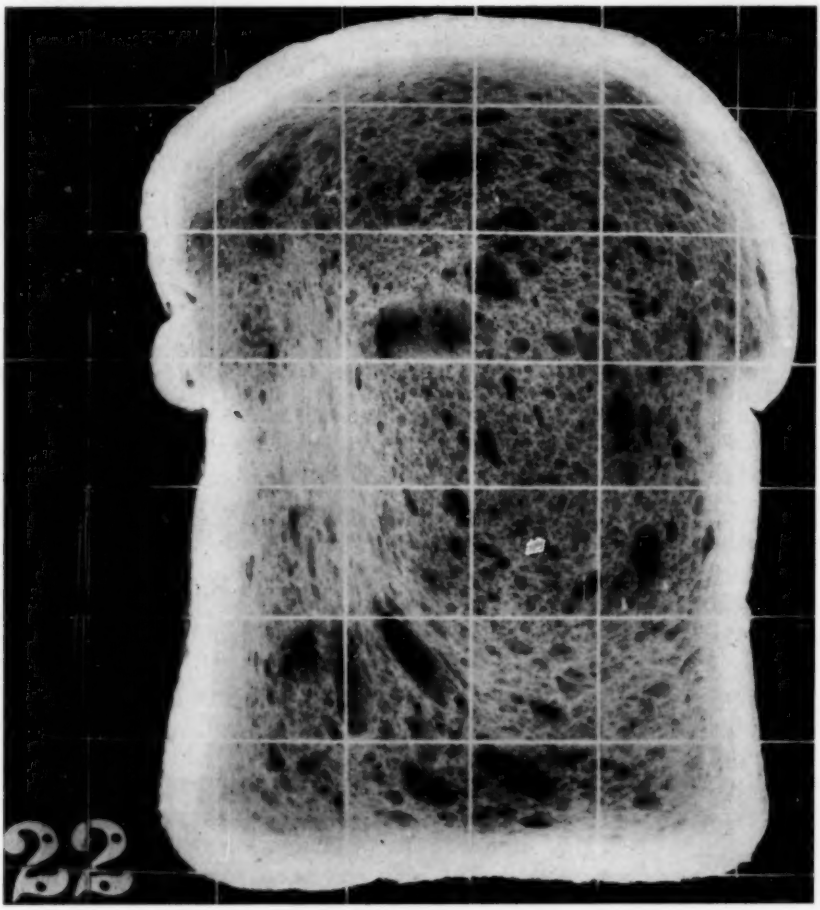

Fig. 1. "Photo-record" of a hand molded loaf of commercial bread, illustrating use of numerals and ¾-in.-mesh screen.

taining the photo-flood bulbs was lined with heavy white paper with a mat surface; a reflector was not used. This light source contributed greatly to the improvement achieved in the pictures of cake. The fogging noticed around the edges of the pound cake shown in Figure 11 of the previous article is mainly due to the relatively long exposure

necessary for the yellow light passing through the yellow cake to affect the photographic paper. By using the photo-flood bulbs this exposure can be shortened so that " fogging " is practically eliminated.

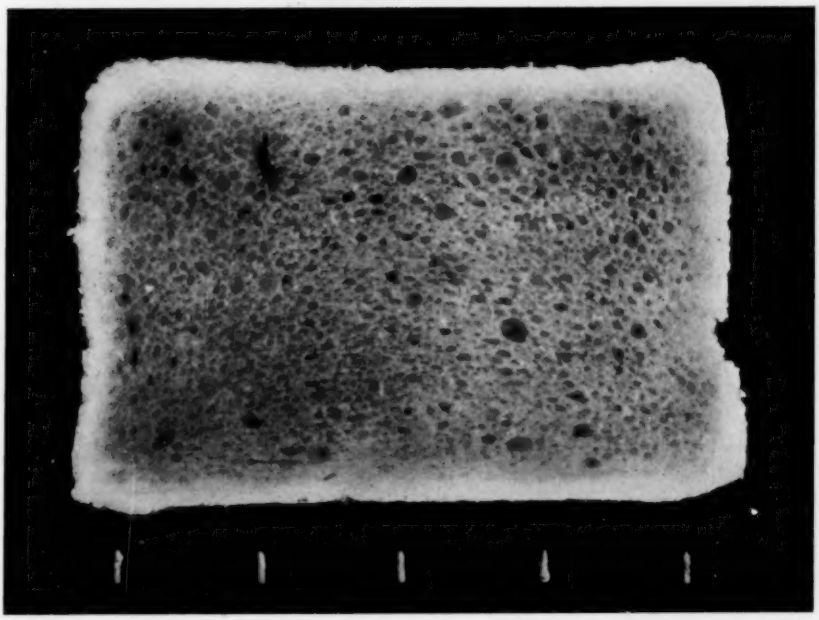

Fig. 2. " Photo-record " of angel food type cake. Thickness of slice 5/16 in. Markings at bottom represent ¼ in.

Photographic Paper

The paper used was Eastman kodabrom, F-No. 2, smooth, glossy, single weight (corresponding papers can be obtained in Agfa and Defender makes). This paper was found to work very well for cakes, including yellow ones, and equally well for bread. Whether this paper is superior to the more " contrasty " papers used for making the pictures in the previous article is perhaps an individual problem. The less contrasty papers yield softer tones and perhaps are more suitable for commercial work. For experimental work, where one wants the defects to be exaggerated, the author favors the contrast papers.

Numbering the " Photo-Records "

The " photo-records " can be numbered simply by placing the proper numeral on the glass tray which holds the slice of bread or cake. The numerals can be cut out of any non-transparent material. The numbers

shown in Figures 1, 3, and 5 are stamped from ordinary galvanized iron, ½ inch high, and are obtainable from a hardward store.

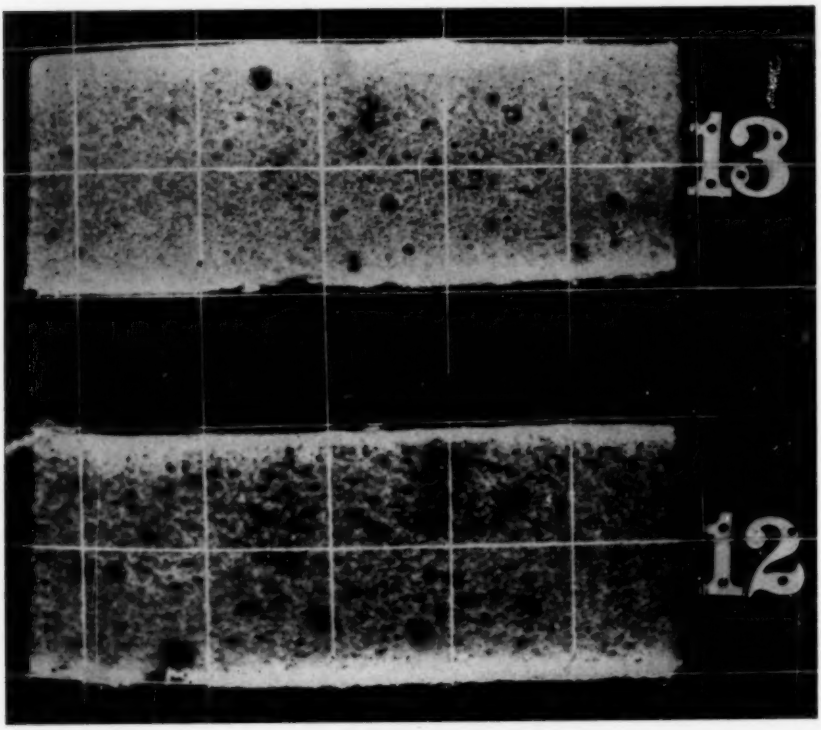

Fig. 3. "Photo-records" of two types of ordinary yellow layer cake. Thickness of slice 5/16 in.

Mesh Screen

A wire-mesh screen placed over the article being photographed is frequently used so that the degree of enlarging or reducing can be determined. Such a screen can be used for "photo-records" also; however, it must be constructed of very fine wire or thread. The one used for the illustrations in this article (Figures 1, 3, and 5) was simply constructed by screening strong thread, No. 25, on a wooden frame by means of tacks.[8] The openings are ¾-inch square. The screen is placed over the slice of bread or cake before photographing. Figure 1 shows this in use with bread.

[8] It has been suggested that finer thread or wire would improve the pictures by producing a network that would not be so prominent.

Cake

With cake, "photo-records" are limited to use with light (white and yellow) cakes. Attempts to apply the method to the darker cakes have not been successful. In order to obtain good pictures it is necessary to have the slice of cake thin. For the more porous cakes, such as angel food, a thickness of $\frac{5}{16}$ inch is about maximum (Fig. 2); for the ordi-

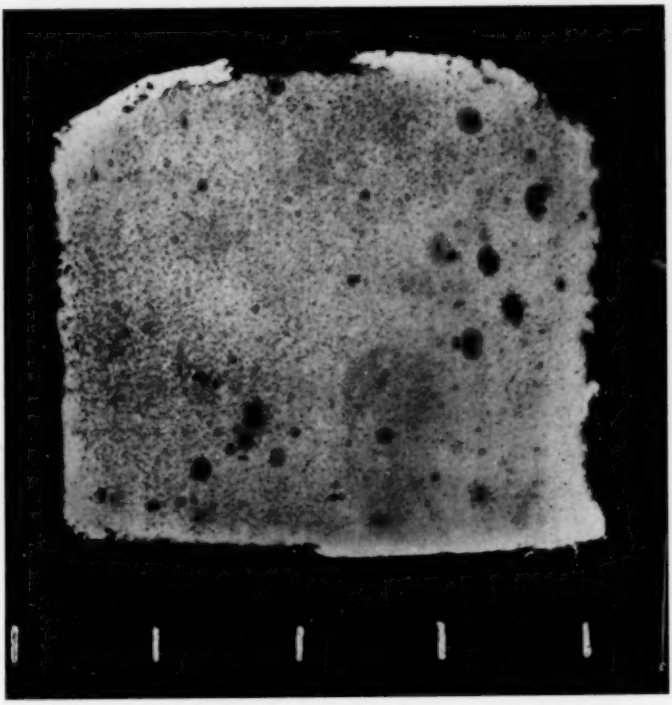

Fig. 4. "Photo-record" of deep yellow pound cake. Thickness of slice ⅛ in. Markings at bottom represent ¾ in.

nary yellow layer, $\frac{3}{16}$ inch is about maximum thickness (Fig. 3); for compact, deep-yellow pound cake, $\frac{1}{8}$ inch is optimum thickness; however, $\frac{3}{16}$ inch will serve fairly well (Fig. 4 and 5); and for white high-ratio layer, $\frac{1}{4}$ inch is about maximum thickness (Fig. 6).

These data on thickness will serve as a guide for most cakes. The limit of thinness to which a cake can be sliced is controlled by its crumbliness; nevertheless, it has been possible to slice almost every cake, of the many tried, thin enough to obtain a good picture. A slicer of the

type used for slicing cold meats was used for slicing the cake; however, a mitre box serves fairly well.

No attempt was made to adjust the thickness of the slices of different cakes so that a uniform time of exposure could be used. Instead, the

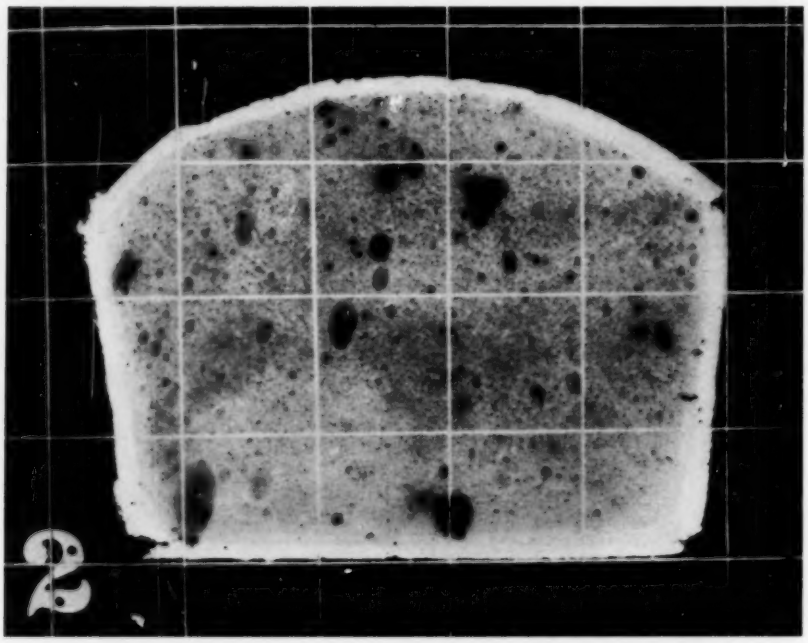

Fig. 5. "Photo-record" of light yellow pound cake. Thickness of slice 3/16 in.

time of exposure was adjusted for each different slice. After some experience this can be determined fairly well by holding the slice up to a constant light source. A photoelectric exposure meter serves very well for this purpose when placed over the slice of cake. It also can be used for bread.[4]

A review of the previous article makes the examples of cake shown here self-explanatory.

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[4] In the case of white bread, a variation of from 3/4 inch to 9/16 inch in the slice thickness made only a small difference in the time of exposure.

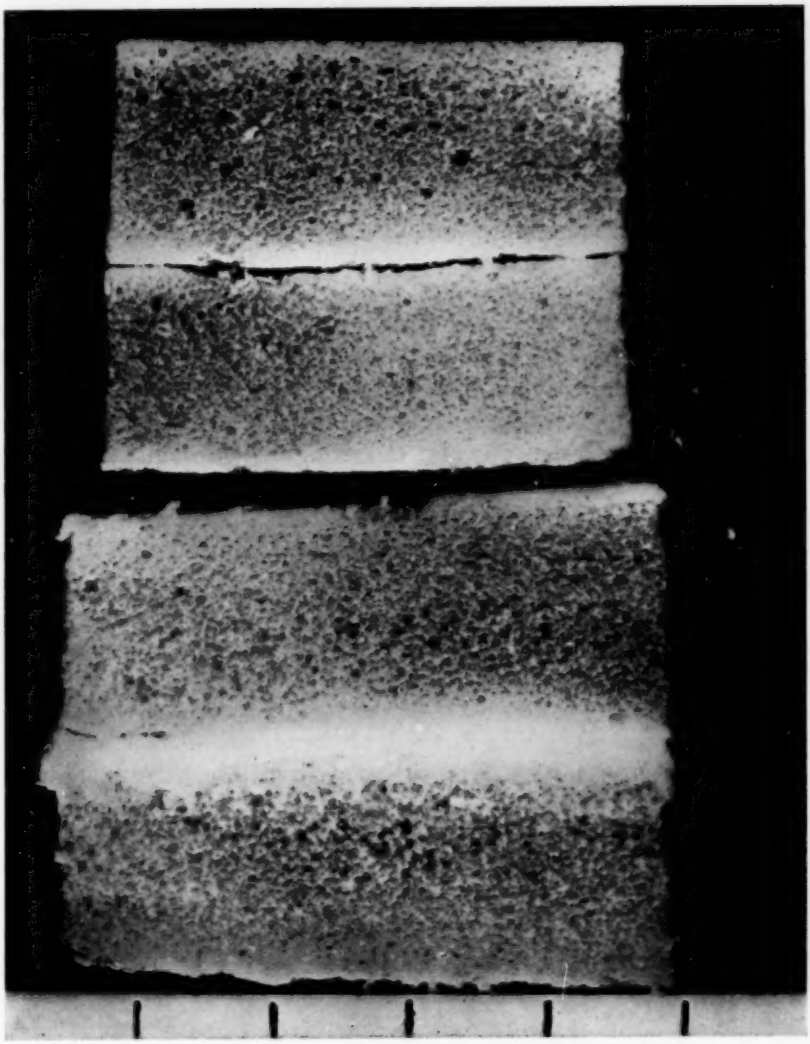

Fig. 6. "Photo-record" of white high-ratio cake. Thickness of slice ¼ in. The light streak in center of the bottom picture is due to dark filling. Markings at bottom represent ¾ in.

Acknowledgment

The author is indebted to several people in the baking and allied industries for reviewing the manuscript.

MODIFICATION OF THE "SWELLING POWER" TEST FOR THE STALING OF BREAD

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Methods of measuring the rate of staling of bread in terms of definite figures are desirable and necessary. Without them it would be impossible to determine with exactness whether special additions to the dough, special dough treatments, etc., help the keeping properties of the resulting bread. The methods already developed are outlined below. A method having ease of operation, reproducibility, and rapidity of obtaining results is to be herewith recommended.

Lehmann (1894) observed that the crumb of bread, on staling, lost some of its power to swell when immersed in water; that is, he found that fresh bread crumb will swell more than stale bread crumb. Katz (1928) states that Balland was the first to show that the swelling power of bread decreases during staling and that this change can be traced to the starch. Katz (1915, 1917, 1928), on the basis of observations of Balland, Lindet (1902), and Lehmann, has used the swelling-power test for the measurement of the staleness of bread, and has recommended it highly.

According to Katz this method of measuring staleness is more satisfactory for general purposes than the method based on measuring the quantity of soluble amylose (1917, 1928), or on measuring the compressibility (termed hardness by Katz) of the crumb (Katz, 1917, 1928; Platt, 1930; and Bailey, 1930). Measurement of the quantity of soluble amylose is time-consuming, and carbohydrates other than those derived from wheat starch interfere to a small extent. Katz's comment on the above methods is as follows: ". . . the method based on determination of the volume of the decantate [swelling power] is the method best adapted to insure safely reproducible figures. All other methods, even if they seem more rational, involve the objection that they easily develop irregularities in the findings."

Katz has worked with the compressibility method quite extensively and Platt (1930) remarks that "Katz discontinued work by this method in favor of the swelling method, which he recommends in preference. Why this is so, is not clear." Platt points out five advantages of the compressibility method; however, the writers agree with Katz that the main disadvantage is the difficulty of obtaining reproducible results.[1] This is due to the fact that any variation in the

[1] Unpublished observations.

cubical size of the piece of crumb, drying of the outside surface of the crumb, dense spots or dense streaks, holes, and temperature cause variations in the results. This means that several loaves must be tested from each batch and the results averaged.

Katz (1934b) [2] has found that bread shows the same changes in x-ray pattern on staling as do starch pastes. The change in the swelling power runs parallel with the changes in x-ray pattern. Thus the swelling-power method, as do the other methods, measures the changes that take place in the starch during staling. It is also pointed out that the x-ray method is more accurate than the swelling-power method as the sediment is often a bit uneven.

Alsberg (1936) reports that Lopin found an improvement in the swelling-power method by using a 25% alcohol solution instead of water. He reports that this causes sedimentation to occur more rapidly and readings are sharper. According to observation by the writers, this procedure only slightly improves the unevenness which often occurs,[3] and judging from the few experiments which were made the variation in the cubic centimeters of sediment between fresh and stale bread is less than when water alone is used.

Katz (1917, 1928) has demonstrated that an excess of water (as is present with the swelling-power method) fixes the degree of staleness of bread; that is, there is no further staling after the bread crumb is in solution. Thus the sediment can be measured on the same sample as many times as is desired. Also, a sample of crumb may be taken when desired, mixed with water in a stoppered flask, and kept until it is convenient to make the determination.

Karacsonyi (1929) has remarked about the method as follows: "Although we get easily measured differences between fresh and stale bread with this method, its chief drawback is that the determination requires a whole day, and, compared with an eventual viscosimetric method, one must concede the preference to the latter."

Karacsonyi prepares his suspension by a method similar to that of Katz; however, instead of letting it stand for 24 hours, he immediately measures its viscosity with an Ostwald viscosity pipette.[4] Accurate control of the temperature is necessary; thus a thermostat must be used. It is stated that the whole determination can be completed in 40 minutes. Only values for fresh bread and bread 48 hours old are given.

After trying this method Katz remarked that at times it is irregular,

[2] J. R. Katz and co-workers, Z. physik. Chem., A, series of papers.

[3] This unevenness is much more evident in bread which has been frozen. Work has been carried on in these laboratories on "frozen bread" and a way of overcoming this unevenness was found in the method described in this paper.

[4] Both this and the Katz swelling-power method are listed in Cereal Laboratory Methods, pp. 86–87, published by A. A. C. C. (1935).

and that it is difficult to determine the relation between the viscosity found and the swelling sought.

Stellar and C. H. Bailey (1938) made a rather extensive investigation of factors which affect the staling of bread. They used the compressibility, sediment volume (swelling power), and viscosity methods concurrently in their investigation. They conclude that, "Compressibility and viscosity measurements of staleness were more consistent and uniform than the data obtained by the sedimentation method. . . . The sedimentation test is not sensitive to minor changes in the condition of the bread and is subject to error due to faulty settling of the crumb."

Although the swelling-power method is accepted as one of the best tests for staleness, Katz, Alsberg (1936a), and L. H. Bailey (1930) have found that it does not show that those procedures of manufacture which the baking trade believes prolongs the life of bread, delay the aging of the starch granules of the crumb. Alsberg comments that "It cannot be doubted that some of these practical procedures do have the effect claimed for them. The explanation probably is that the sedimentation test tells the state of the starch correctly but that this is probably not the whole story."

However, until a better method is developed, the swelling-power method will undoubtedly continue to meet with favor.

The swelling-power method, as described by Katz, has been used in this laboratory. Trouble has been encountered in getting the crumb through the fine bolting cloth, especially in that the cloth breaks very easily with continued rubbing and often the sediments are so uneven that accurate readings cannot be made. Variations of as much as 6 cc. have been noted at times between the maximum and minimum of the sediment surface, with an average of about 4 cc. In order to shorten the time necessary for a determination and minimize the above-mentioned disadvantages, the following work was undertaken.

Experimental

Katz, in a popularly written article (1934c) mentions that a baker can use a metal sieve instead of the bolting cloth. A brass-frame, 200-mesh sieve 5 inches in diameter has been found to work satisfactorily. The sieve has 78.7 openings per centimeter, and the bolting cloth recommended by Katz has 80 openings per centimeter. The sieve has the advantage of fitting snugly on top of a two-liter pyrex beaker, which serves to catch the washings. The use of the sieve in general has been much more satisfactory than the bolting cloth; in addition the sieve is durable and saves considerable time. However,

this procedure did not eliminate the unevenness in the sediment which often developed.

Since the method is based on sedimentation there seemed to be no reason why the centrifuge would not be helpful in correcting this unevenness. Katz (1934d) has mentioned the use of the centrifuge but does not tell how he used it. However, he remarks: "The method . . . did not work as satisfactorily [as the ordinary method] and was much more complicated." Nevertheless, the writers have experimented with it and have found that the centrifuge can be used satisfactorily.

The wheel of the centrifuge had a diameter of 10½ inches. A few preliminary experiments indicated that a speed of 1400 r.p.m. would be very satisfactory. This speed has been used throughout the work. The effect of temperature was investigated and variations from 18° to 26° C. were without effect on the results. Two standard tubes were tried:

A A 30-cc. conical sediment tube graduated in 0.1-cc. divisions from 0 to 3 cc., in 0.2-cc. divisions from 3 cc. to 10 cc., and in 0.5-cc. divisions from 10 cc. to 30 cc.

B A 50-cc. conical sediment tube graduated in 0.5-cc. divisions from 0 cc. to 10 cc. and 1.0-cc. divisions from 10 cc. to 50 cc.

The tubes must be thoroughly clean and dry before filling. They are filled from the suspension in the 250-cc. graduate (used in Katz method) after it has been made up to the mark. The solution in the graduate must be shaken thoroughly just prior to filling the sediment tube.

The effect of time of centrifuging on the volume of sediment was studied first. Some typical results are given in Table I and plotted in Figure 1. The sediment in Tube *A* (30-cc. tube) is more easily measured because of the finer degree of calibration. The surface of the sediment when the 30-cc. tube was used was even and did not tend to develop irregularities as easily as with the 50-cc. tube. Since the amount of sediment drops to a minimum and ceases to decrease, determinations on the rate of staling should be made at a time when the amount of sediment is not varying. This was not done because in this region the surface of the sediment had a tendency at times to become uneven, especially with the 50-cc. tube. It seemed better to centrifuge for 2 or 4 minutes and control the time of centrifuging as accurately as possible. Since there is a more rapid change between the second and fourth minutes of centrifuging in the amount of sediment with the 50-cc. tube than with the 30-cc. tube, the 30-cc. tube is recommended. Nevertheless, the 50-cc. tube can be used and typical results are given in Table II and plotted in Figure 2.

TABLE I

EFFECT OF TIME CENTRIFUGED ON CUBIC CENTIMETERS OF SEDIMENT AT 4, 24, AND 70 HOURS OUT OF OVEN (WHITE BREAD, MEDIUM FORMULA, WRAPPED IN WAX)

Age of bread	Time in centrifuge	Sediment, tube A		Sediment, tube B	
		Separate determinations	Average	Separate determinations	Avergae
Hrs.	*min.*	*cc.*	*cc.*	*cc.*	*cc.*
4	2	2.70, 2.72	2.71	4.50, 4.30	4.40
4	4	2.60, 2.60	2.60	4.30, 4.20	4.25
4	6	2.52, 2.55	2.54	4.00, 4.15	4.08
4	8	2.50, 2.49	2.50	3.95, 4.10	4.03
4	10	2.50, 2.50	2.50	3.90, 4.10	4.00
4	12	2.50, 2.50	2.50	3.90, 4.10	4.00
24	2	2.30, 2.30	2.30	3.65, 3.80	3.73
24	4	2.20, 2.25	2.23	3.50, 3.65	3.58
24	6	2.15, 2.20	2.18	3.50, 3.60	3.55
24	8	2.15, 2.20	2.18	3.45, 3.55	3.50
24	10	2.12, 2.18	2.15	3.45, 3.55	3.50
24	12	2.10, 2.13	2.12	3.45, 3.55	3.50
70	2	2.10, 2.19	2.15	3.50, 3.35	3.43
70	4	2.05, 2.10	2.08	3.50, 3.30	3.40
70	6	2.00, 2.09	2.05	3.45, 3.25	3.35
70	8	2.00, 2.07	2.04	3.42, 3.24	3.33
70	10	2.00, 2.03	2.02	3.40, 3.22	3.31

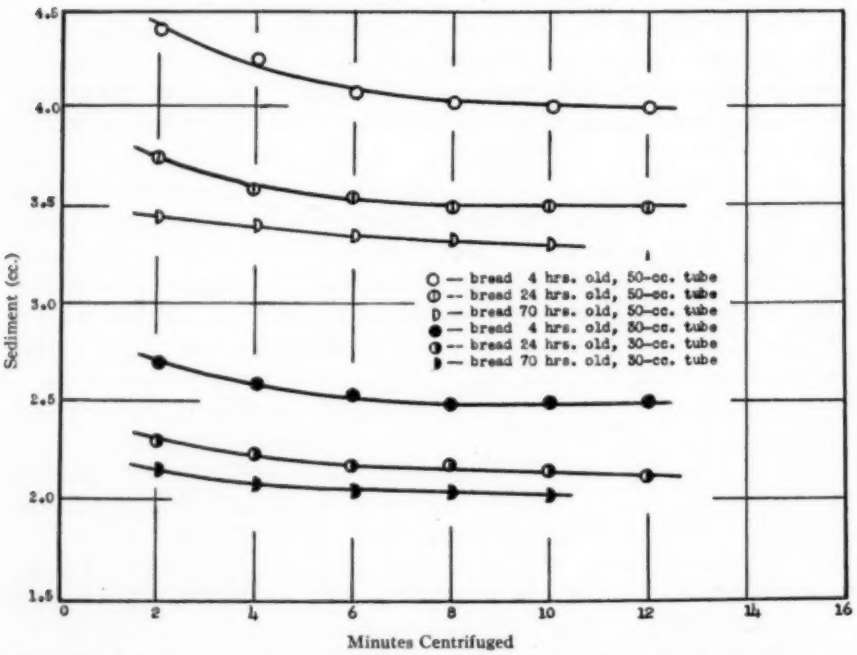

Fig. 1. Effect of time centrifuged on cubic centimeters of sediment at 1400 r.p.m.—bread of various ages, sediment tubes of two sizes.

TABLE II

EFFECT OF AGE OF BREAD NO. 2 (WHITE BREAD, WRAPPED IN WAX, MEDIUM-RICH FORMULA) ON CUBIC CENTIMETERS OF SEDIMENT (50-CC. TUBE USED)

Age of bread	Sediment, 4 min. in centrifuge		Sediment, 8 min. in centrifuge	
	Separate determinations	Average	Separate determinations	Average
Hrs.	*cc.*	*cc.*	*cc.*	*cc.*
¼	4.95, 4.95, 4.95, 4.95	4.95	4.75, 4.75, 4.75, 4.75	4.75
2½	4.80, 4.80	4.80	4.55, 4.55	4.55
7	4.50, 4.50	4.50	4.25	4.25
19¼	3.75, 3.75	3.75	3.65, 3.65	3.65
26	3.60, 3.65, 3.55	3.60	3.50, 3.60, 3.50	3.53
46	3.45, 3.50	3.48	3.40, 3.48	3.44
68	3.30, 3.30	3.30	3.25, 3.25	3.25

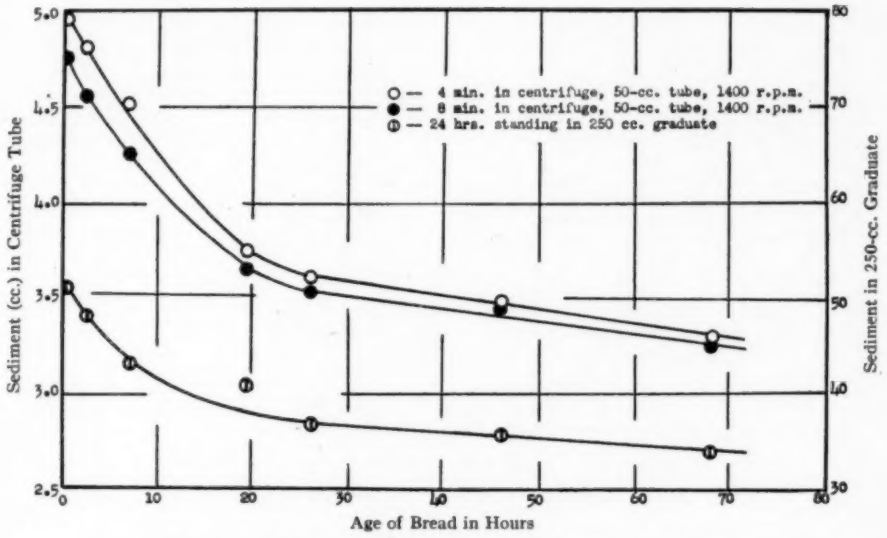

Fig. 2. Effect of age of white bread (medium-rich formula) on sediment.

It will be noted that the results of consecutive determinations agree exceptionally well; however, it must be remembered that this tube cannot be read as accurately as the 30-cc. tube. All the remaining results reported were obtained with the 30-cc. tube. Table III and Figure 3 give typical results obtained for white bread made from a rather rich formula. Determinations also have been made on rye bread. Table IV and Figure 4 are for rye bread (15% dark rye flour). Sediment measurements for the breads reported in Tables II, III, and IV were also made by the Katz method and are reported in Table V. The results are plotted along with the corresponding centrifuge measurements.

TABLE III

EFFECT OF AGE OF BREAD NO. 3 (WHITE BREAD, UNWRAPPED, RICH FORMULA) ON CUBIC CENTIMETERS OF SEDIMENT (30-CC. TUBE USED)

Age of bread	Sediment, 2 min. in centrifuge		Sediment, 4 min. in centrifuge	
	Separate determinations	Average	Separate determinations	Average
Hrs.	*cc.*	*cc.*	*cc.*	*cc.*
¾	3.00, 2.90	2.95	2.80, 2.75	2.78
4	2.70, 2.70	2.70	2.55, 2.55	2.55
7	2.65, 2.65	2.63	2.50, 2.50	2.50
9½	2.60, 2.59, 2.58, 2.59	2.59	2.45, 2.46, 2.46, 2.44	2.45
12	2.50, 2.50, 2.51, 2.51	2.51	2.35, 2.35, 2.35, 2.37	2.36
26	2.30, 2.30, 2.30, 2.32	2.30	2.20, 2.22, 2.20, 2.15	2.19
47	2.20, 2.10, 2.20, 2.10	2.15	2.09, 2.10, 2.10, 2.18	2.12
70	2.10, 2.19	2.15	2.10, 2.05	2.08

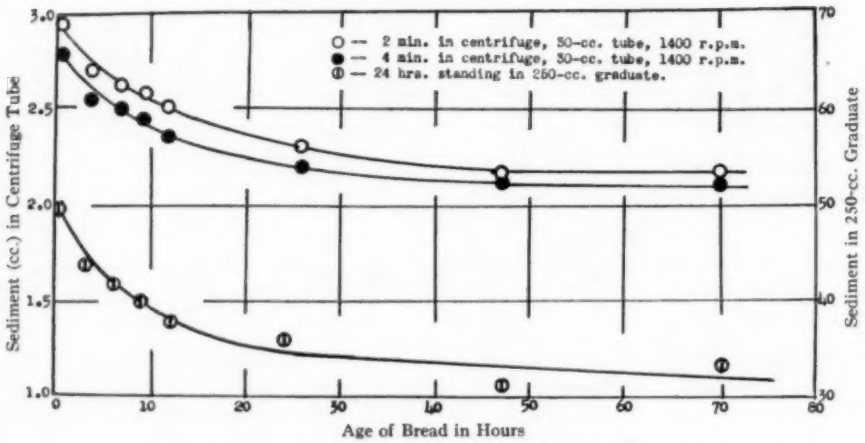

Fig. 3. Effect of age of white bread (rich formula) on sediment.

Throughout the figures it will be noted that the curves determined according to Katz are similar in shape to those determined by the centrifuge method, especially those made with the 30-cc. tube. In general, then, the rate of staling is undoubtedly the same when recorded by either method.

It appears that more points fall off the curve when determined by the Katz method than with the centrifuge method. However, it should be emphasized that all of the results representing one determination were taken from the same loaf of bread. Determinations at different ages of bread were made on separate loaves from the same batch. It was thought that the accuracy might be improved by using sediments from several different loaves (same age, from the same

TABLE IV

EFFECT OF AGE OF BREAD NO. 4 (RYE BREAD, 15% DARK RYE, UNWRAPPED) ON CUBIC CENTIMETERS OF SEDIMENT (30-CC. TUBE USED)

Age of bread	Sediment, 2 min. in centrifuge		Sediment, 4 min. in centrifuge	
	Separate determinations	Average	Separate determinations	Average
Hrs.	*cc.*	*cc.*	*cc.*	*cc.*
¾	3.05, 3.00	3.03	2.85, 2.80	2.83
4	2.88, 2.89	2.89	2.70, 2.70	2.70
7	2.80, 2.79	2.80	2.67, 2.66	2.67
9½	2.75, 2.75, 2.75, 2.76	2.75	2.62, 2.63, 2.61, 2.63	2.62
12	2.68, 2.69	2.69	2.53, 2.53	2.53
26	2.50, 2.50, 2.50, 2.48	2.50	2.30, 2.35, 2.30, 2.30	2.32
47	2.38, 2.32, 2.30, 2.32	2.33	2.29, 2.29, 2.20, 2.22	2.25
70	2.25, 2.30, 2.29, 2.30	2.29	2.20, 2.15, 2.20, 2.20	2.19

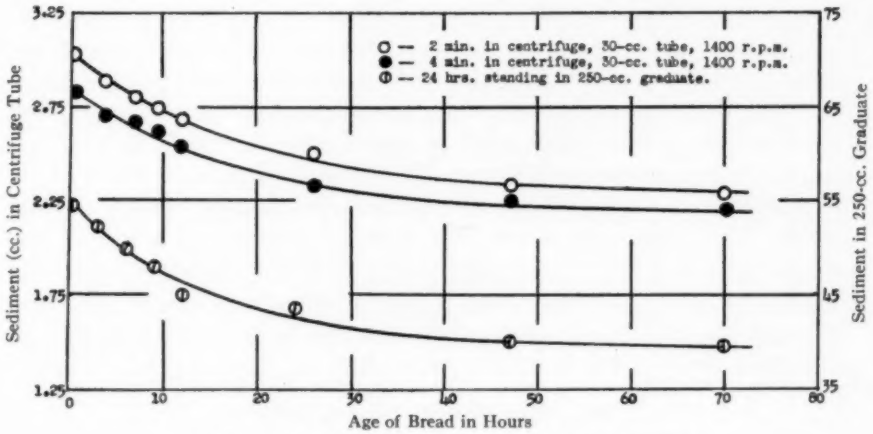

Fig. 4. Effect of age of rye bread (15% dark rye) on sediment.

TABLE V

SEDIMENT (AVERAGE TWO DETERMINATIONS) DETERMINED AS PER KATZ FOR BREADS GIVEN IN TABLES II, III, AND IV

Bread No. 2 (Table II)		Bread No. 3 (Table III)		Bread No. 4 (Table IV)	
Age	Sediment	Age	Sediment	Age	Sediment
Hrs.	*cc.*	*Hrs.*	*cc.*	*Hrs.*	*cc.*
⅓	51.0	¼	50.0	¼	54.5
2½	48.0	3	44.0	3	52.5
7	43.0	6	42.0	6	50.0
19¼	41.0	9	40.0	9	48.0
26	37.0	12	38.0	12	45.0
46	36.0	24	36.0	24	43.5
68	34.0	47	31.0	47	40.0
		70	33.0	70	39.5

batch) and the results averaged as was done by Platt (1930) in connection with compressibility measurements. A few experiments indicated that this was true in a few cases, especially when the Katz method was used. However, reliable results can be obtained by making up only one preparation in the 250-cc. graduate and then transferring two aliquots of this into the centrifuge tubes.

Considering the results from the 30-cc. tube or from the Katz method, it is seen that the type of curve depends on the type of bread. The curves for the rye bread start just as high and fall off less gradually than those for white bread, thus substantiating the fact that rye bread stales less rapidly than white. The results herein obtained using the Katz method are very similar to those reported by Katz himself.

It has been mentioned that irregularities in the surface of the sediment with the Katz method (using 250-cc. graduate) have been 4 cc. on the average. Assuming that the correct result can be approximated to within 2 cc. and considering the maximum amount of sediment to be 50 cc., we see that this introduces an error of 4%. Assuming that this is the only reason for variations in separate determinations, the minimum error that would often be present would be 4%.

In the centrifuge modification, there is practically no error due to irregularities in the surface of the sediment, for the surfaces are almost always even after centrifuging for 2 or 4 minutes. However, from the tables it will be seen that the maximum variation in readings with the 30-cc. tube is 0.1 cc. Considering the maximum amount of sediment to be 3.00 cc., the maximum error is 3.3% as compared to a minimum error of 4% for the Katz method.

The Modified Method in Brief

Ten grams of bread crumb, taken from near the center of the loaf, is weighed to the nearest 0.1 gram and placed on a brass-frame, 200-mesh sieve of 5 inches in diameter; the sieve in turn is placed on a 2-liter pyrex beaker which serves to catch the washings. The bread is moistened with water [5] at 20 ± 2° C. The sieve is transferred to a second beaker and the bread rubbed through with the forefinger and the second finger. The washings from the first beaker are used to moisten the crumb as the rubbing is continued. Water is added and rubbing is continued until all of the bread has passed through the sieve. The sieve is then placed back on the first beaker and the entire mass is washed back through the sieve. Care must be taken that the volume is not over 250 cc. The suspension is then transferred to a 250-cc. graduate [6] and the solution made up to 250 cc. The graduate is

[5] Ordinary tap water of about medium hardness has been the most satisfactory in many cases. This is especially true of bread which has been frozen.

[6] Three drops of toluene should be added to prevent fermentation if the sediment suspension is not to be centrifuged immediately.

shaken well and a thoroughly clean centrifuge tube (type A, 30 cc., described above) is filled immediately. The tube is then centrifuged for 2 to 4 minutes [7] (time must be constant) at 1400 r.p.m., when wheel of centrifuge is 10½ inches in diameter. The time and speed can be altered and standardized for wheels of different diameters.

Advantages and Uses

The above modifications of the "swelling-power" method offer advantages in that the modified method is more convenient, requires less time for a determination (a determination can be made in about 30 minutes as compared to 24 hours for the Katz method), readings can be made more accurately, and the results are very reproducible.

For the benefit of readers other than technicians, the authors wish to point out that one important consideration in any physical or chemical method of measuring staleness is its correlation with actual consumer judgment. Although these methods are of great importance, since they eliminate the human element, a few researches in the past have shown that results obtained by some of these methods do not agree with those of human beings. Reservation is made, pending further research, as to whether the modified method described in this paper gives measurements of staleness which can be correlated with the results of human observers.

It is hoped that this method will enable baking technologists to more easily tell the baker what ingredients, what processes of baking, and what methods of storage will keep his products fresh for the longest periods of time.

Summary

Various methods for determining the staleness of bread have been enumerated. The swelling-power method has been modified in that a centrifuge is used in determining the amount of sediment and a 200-mesh sieve is used instead of bolting cloth. This makes the method more convenient and it requires less time.

Acknowledgment

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[7] Readings may be taken at both 2 and 4 minutes which when compared with standard curves for 2 and 4 minutes will serve as a check on each other.

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THE BIOLOGICAL VALUE OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS [1]

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Proteins are not of the same value in nutrition because of the variations in their amino acid content, and the importance of the quality of proteins is universally recognized. A quantitative measure of protein values is obtained by determining the biological value, for which estimation several methods, such as nitrogen balance, growth, or nitrogen storage, are available.

Mitchell and Hamilton (1929) state that values obtained in experiments with pigs and chickens are quite similar to those obtained with rats. The ruminants are excepted, because the microörganisms of the paunch feed on the non-protein nitrogenous material and distinctly modify its biological value.

The purpose of this investigation was to evaluate the proteins of whole brown rice, white polished rice, rice bran, and rice polish for the functions of maintenance and growth.[2] The material was purchased from a local mill and was supposed to be representative of products for human consumption and for feeding pigs and poultry.

A study of the literature revealed that Mitchell (1924), employing the nitrogen-balance method, found an average biological value of 86 for whole rice fed at a 5% protein level, and of 68 for rice bran at a 10% protein level.

Tsan-Wen Li (1930) reported an average value of 79 for a Chinese variety fed at a 10% protein level, and quite recently Basu and Basak (1937) found a value of 80 for polished rice of the Aman variety and also of the Aus variety. Their polishings had an average value of 69 and 68 respectively, at a 5% protein level.

In this paper results obtained with a growth and with a nitrogen-balance method are reported, with the rat as the experimental animal. The compositions of the diets used for these experiments are given in Table I.

Growth Experiments

A growth comparison of the relative protein values of whole rice and white polished rice at a 5.5% protein level (ration Nos. 1 and 2,

[1] Research paper No. 480, Journal Series, University of Arkansas.
[2] Blue Rose variety obtained from the Cooperative Rice Growers' Assoc., Stuttgart, Ark.

Table I) was made by the modified paired-feeding method of Mitchell, Burroughs, and Beadles (1936). The principle of Osborne and Mendel (1916) that "the only strict basis for comparisons is afforded by experiments in which the animals receive the same amount of food during the same period of time and make the same gains in weight," has been used in his technique. This was accomplished through the use of a nitrogen-free ration (No. 12, Table I), varying amounts of which were fed with the protein ration that was better utilized.

TABLE I

COMPOSITION OF THE RATIONS

Number and name of ration	N	Salt mixture[1]	Butterfat	Cellu flour	Dried egg	Starch	Purified sucrose
	%	%	%	%	%	%	%
1. Whole rice (77.0%)	0.92	4.0	8.0	—	—	11.0	—
2. Polished rice (88.0%)	0.92	4.0	8.0	—	—	—	—
3. Rice bran (67.0%)	1.32	4.0	8.0	—	—	21.0	—
4. Rice polishings (65.0%)	1.32	4.0	8.0	—	—	23.0	—
5. Casein (7.0%)	0.93	4.0	10.0	4.0	—	65.0	10.0
6. Rice polishings (37.0%)	0.92	4.0	10.0	—	—	39.0	10.0
7. Rice bran (34.0%)	0.92	4.0	10.0	—	—	42.0	10.0
8. Whole-milk powder (31.2%)	1.31	4.0	10.0	4.0	—	40.8	10.0
9. Skimmilk powder (23.7%)	1.31	4.0	10.0	4.0	—	48.3	10.0
10. Polished-rice protein extract (51.0%)	1.36	4.0	10.0	4.0	—	21.0	10.0
11. Standardizing ration	0.60	4.0	10.0	4.0	4.0	68.0	10.0
12. Nitrogen-free ration	—	4.0	10.0	4.0	—	72.0	10.0

[1] Osborne and Mendel No. 351.

Nine pairs of rats were used in this comparative study. Litter mates of the same weight and sex were employed, the initial weight being about 50 grams. Individual food-consumption records were kept and the animals were weighed twice a week, when adjustments were made for the intake of the nitrogen-containing ration and the ration that was nitrogen free. The nine pairs of rats were killed at the end of 130 days and their body length from mouth to anus was measured. Similar measurements were made at the beginning of the experiments. Differences in increase in body length might be found between the two growth-promoting proteins. The results of this paired-feeding experiment are given in Table II.

In all nine pairs the whole-rice-fed rats utilized their food better than the polished-rice-fed ones, and less nitrogen-containing food was needed by the former for the same gain in weight as was obtained by the latter. A certain intake of nitrogen from the whole-rice ration promoted the

TABLE II

A COMPARISON OF THE GROWTH-PROMOTING VALUE OF THE PROTEINS OF WHOLE RICE AND POLISHED RICE BY A MODIFIED PAIRED-FEEDING METHOD

Duration 130 days, 5.5% protein intake.

Pair No.	Rat No. and sex	Ration	Increase in body weight	Increase in body length	Food intake	Nitrogen intake	Ratio	Protein (N × factor)[1] intake	Gain per gram of protein intake
			g.	mm.	g.	g.		g.	g.
1	1 ♀	Whole rice	72	45	866 (50)[2]	7.95	1.00	47.30	1.52
	2 ♀	Polished rice	72	45	916	8.45	1.06	50.27	1.43
2	3 ♀	Whole rice	99	56	965 (80)	8.73	1.00	50.94	1.94
	4 ♀	Polished rice	99	50	1045	9.63	1.10	57.29	1.73
3	5 ♀	Whole rice	87	48	883 (80)	8.16	1.00	48.55	1.79
	6 ♀	Polished rice	87	48	965	8.88	1.09	52.83	1.65
4	7 ♂	Whole rice	82	45	827 (70)	7.64	1.00	45.46	1.80
	8 ♂	Polished rice	82	41	897	8.27	1.08	49.21	1.67
5	9 ♂	Whole rice	101	41	977 (100)	9.02	1.00	53.67	1.88
	10 ♂	Polished rice	101	41	1077	9.93	1.10	59.08	1.71
6	11 ♂	Whole rice	102	40	975	9.01	1.00	53.61	1.91
	12 ♂	Polished rice	102	40	1040	9.59	1.06	57.06	1.79
7	13 ♂	Whole rice	94	52	900 (50)	8.31	1.00	49.44	1.90
	14 ♂	Polished rice	94	46	950	8.76	1.05	52.12	1.80
8	15 ♂	Whole rice	94	42	965 (110)	8.91	1.00	53.01	1.77
	16 ♂	Polished rice	94	41	1075	9.91	1.11	58.96	1.61
9	17 ♂	Whole rice	83	41	925 (50)	8.55	1.00	50.87	1.63
	18 ♂	Polished rice	83	41	975	8.99	1.05	53.49	1.55
	Average, whole rice		—	—	920 (73)	8.47	1.00	—	1.80
	Average, polished rice		—	—	993	9.15	1.08	—	1.66

[1] Factor for rice 5.95.
[2] Amount of nitrogen-free ration consumed.
Mean difference (M), 0.140; standard deviation of differences (S), 0.042; ratio M/S, 3.3; odds, 9,999 : 1; probability, 0.00001.

same gain in weight as a larger intake of polished-rice nitrogen. The ratios between the nitrogen intake of pair mates (Table II, eighth column) indicate that one gram of nitrogen from the whole rice ration was as effective in promoting the functions of maintenance and growth as an average of 1.08 g. of nitrogen from the polished-rice ration. The average gain in weight per gram of protein intake was 1.80 for the animals fed the whole-rice ration and 1.66 grams for those fed the polished-rice ration, with a statistically significant difference of 0.14 gram. Analyzed by Student's method (1908), the probability that chance alone determined the outcome is 0.00001 ($M = 0.14$, $S = 0.042$) with reference to the difference between the average gains obtained. A

TABLE III

A COMPARISON OF THE GROWTH-PROMOTING VALUE OF THE PROTEINS OF RICE BRAN AND RICE POLISH BY A MODIFIED PAIRED-FEEDING METHOD

Duration 112 days, 8% protein intake.

Pair No.	Rat No. and sex	Ration	Increase in body weight	Increase in body length	Food intake	Nitrogen intake	Ratio	Protein (N × factor)[1] intake	Gain in weight per gram of protein intake
			g.	mm.	g.	g.		g.	g.
10	19♂	Rice bran	129	45	991	13.03	1.14	78.52	1.64
	20♂	Rice polishings	129	51	871 (120)[2]	11.45	1.00	68.12	1.89
11	21♂	Rice bran	125	51	1120	14.72	1.25	87.58	1.43
	22♂	Rice polishings	125	51	895 (225)	11.77	1.00	70.03	1.78
12	23♂	Rice bran	140	47	1018	13.38	1.12	79.61	1.76
	24♂	Rice polishings	140	51	908 (110)	11.94	1.00	71.04	1.97
13	25♀	Rice bran	103	49	900	12.23	1.30	72.76	1.42
	26♀	Rice polishings	103	49	765 (195)	9.40	1.00	55.93	1.84
14	27♀	Rice bran	124	53	1084	14.25	1.13	84.78	1.46
	28♀	Rice polishings	124	53	954 (130)	12.54	1.00	74.61	1.66
15	29♀	Rice bran	111	47	1030	13.54	1.13	80.56	1.38
	30♀	Rice polishings	111	49	910 (120)	11.96	1.00	71.16	1.56
16	31♂	Rice bran	93	45	900	11.83	1.34	70.38	1.32
	32♂	Rice polishings	93	45	670 (230)	8.81	1.00	52.41	1.77
17	33♀	Rice bran	122	45	1100	14.46	1.22	86.03	1.42
	34♀	Rice polishings	122	46	900 (200)	11.83	1.00	70.38	1.73
18	35♂	Rice bran	129	55	1082	14.23	1.21	84.66	1.52
	36♂	Rice polishings	129	57	892 (190)	11.73	1.00	69.79	1.85
	Average, rice bran		—	—	1032	13.57	1.20	—	1.48
	Average, rice polishings		—	—	863 (169)	11.35	1.00	—	1.79

[1] Factor for rice 5.95.
[2] Amount of nitrogen-free ration consumed.
Mean difference (M), 0.31; standard deviation of differences (S), 0.097; ratio M/S, 3.2; odds, 9,999 : 1; probability, 0.00001.

probability smaller than 0.03 is according to current biometrical practice a criterion of high significance. In four pairs the increase in body length was greater in the whole-rice-fed animals; in the other five pairs the increase was the same for both rats.

A similar comparative study was made of the growth-promoting value of the proteins of rice bran and rice polishings, by exactly the same technique as described above. The protein level was 8% (rations 3 and 4, Table I) and the experiment lasted 112 days. The results of this experiment are given in Table III, from which it can be seen that

in all nine pairs the rice-polish-fed rats needed less nitrogen to promote the same gain in weight than the rice-bran-fed ones. The ratios between the nitrogen intake of pair mates (Table III, eighth column) indicate that one gram of nitrogen from the rice polishings ration was as effective in promoting the functions of maintenance and growth as an average of 1.20 grams of nitrogen from the rice-bran ration. The average gain in weight per gram of protein consumption was 1.79 g. for the animals fed the rice-polish ration and 1.48 g. for those fed the rice-bran ration. The difference of 0.302 g. between these gains was found to be statistically significant when Student's method for the statistical analysis of paired experimental observations was applied ($M = 0.31$; $S = 0.097$; $P = 0.00001$). In five pairs the increase in body length was greater in the rice-polish-fed rats; the increase was the same in the remaining four pairs.

Metabolism Experiments

Differences in the growth-promoting values of the rice proteins as revealed by the modified paired-feeding method can be explained with the aid of metabolism experiments in which digestibility coefficients and biological values are obtained. The metabolism method of Mitchell (1923) was used in these nitrogen balance studies. Young rats of an initial weight of 60 to 70 g. were employed for the determination of the biological value of rice and its by-products at 5% and 8% protein levels. The experiment consisted of three periods and the test animals were divided into two groups with 4 or 5 animals in a group.

In the first period the test rations were fed to two different groups and in the third period the test rations were reversed for these two groups, while both groups received the standardizing ration (No. 11, Table I) in the second period.

The results of these nitrogen balance studies are summarized in Table IV. Biological values obtained for whole-milk powder and skim-milk powder, casein, and lactalbumin are included for comparison. It can be seen that although whole rice had a somewhat lower digestibility than polished rice (96.5 against 98.0), the biological value of the latter is lower than that of the former, resulting in a better utilization of the whole-rice protein at a 5% level of protein intake and a better rate of growth.

Both digestibility and biological value are lower for the rice bran, compared with those for rice polish at an 8% level of protein intake. Rice polish is better utilized and promotes better growth than rice bran as a result of the greater losses of the nitrogen of rice bran during digestion and metabolism.

TABLE IV

DIGESTIBILITIES AND BIOLOGICAL VALUES OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS AT TWO PROTEIN LEVELS

Protein	Number of determinations	Average true digestibility	Average biological value
5–6% whole rice	48	96.5±.29	72.7±.34
5–6% polished rice	30	98.0±.18	66.6±.18
5% rice bran	8	77.6±.66	84.9±.63
5% rice polishings	18	91.3±.40	82.9±.96
5% casein	8	98.2±.49	81.5±1.00
8% rice polishings	10	88.7±.60	78.9±.97
8% rice bran	10	83.0±.64	71.9±.78
8% whole milk powder	10	93.5±.41	85.6±1.05
8% skim milk powder	10	93.9±.57	87.8±.71
8% casein [1]	12	99.0±.22	69.5±1.00
8% lactalbumin [1]	15	98.0±.40	84.0±.60
9% polished rice protein extract	8	78.3±.48	66.2±.51

[1] Data from: Digestibility, Metabolism, and Nutritive Value of Lactalbumin, by M. C. Kik, University of Arkansas Bulletin No. 352.

The biological values of rice bran and rice polish at a 5% protein level are higher than those of the whole kernel and of the rice flour and compare very favorably with that obtained for casein at that protein level.

At an 8% level of protein intake, the values are somewhat lower for the proteins of rice bran and rice polish than those obtained for whole-milk powder, skimmilk powder, and lactalbumin, all of which are known to possess a high nutritive value.

Summary

A growth comparison of the relative protein values of whole rice and white polished rice fed rats at a 5.5% protein level and of rice bran and rice polishings at an 8% protein level was made using a modified paired feeding method. It was found that 1 g. of nitrogen from the whole-rice ration was as effective in promoting the functions of maintenance and growth as an average of 1.08 g. of nitrogen from the polished-rice ration. One gram of nitrogen from the rice-polishings ration was as effective as 1.20 g. of nitrogen from the rice-bran ration. The average gain in weight per gram of protein intake was 1.80 g. for the rats fed whole rice and 1.66 g. for those fed the white polished rice, at a 5.5% protein level. These figures were 1.79 g. for rats fed rice polishings and 1.48 g. for rats fed rice bran at an 8% protein level.

The biological values (Mitchell's method) for whole rice, white polished rice, rice bran, rice polishings, and casein at a 5% protein level were 72.7, 66.6, 84.9, 82.9, and 81.5 respectively. These values were 85.6, 87.8, 69.5, 84.0, 78.9, and 71.9 for whole-milk powder, skimmilk powder, casein, lactalbumin, rice polishings, and rice bran respectively, at an 8% protein level.

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BOOK REVIEW

Untersuchungsmethoden für Brotgetreide, Mehl und Brot. By Paul Pelshenke. Published by Moritz Schafer, Leipzig, Germany. 288 pages. Price R.M. 11.5.

This manual includes a vast amount of material in relatively small space. The analytical and other methods are classified under 163 sections or divisions and many of these are further subdivided to include several methods designed for the same general purpose.

Sampling methods are presented in the first section, followed by a series of physical tests of cereals. Purity, germinating power, and various forms of damage are then covered. Methods for the determination of ash, protein (including the individual proteins of wheat), gluten quantity and quality, fermentation and enzymes, physical properties of dough, carotin and color, acidity and pH, carbohydrates, fats, fiber and pentosans are presented in the order named. This is followed by the description of many special methods adapted to cereals (bread and other cereal products, as well as baking powder), identification of impurities and mixtures, presence of special ingredients, and conformity of products to standards of quality. Evaluation of bread quality is discussed, together with score cards for various bread types. A series of 39 tables is provided which include equivalents of hectoliter weight in pounds per bushel, sugar conversion tables, buffer solutions, sieve dimensions, relative humidity, and other pertinent data.

The book is least complete in the departments devoted to physical and physicochemical methods. Thus the electrical methods for the estimation of moisture content are dismissed with about eight lines of text. Mechanical dough testing is discussed at some length (about a dozen pages) but no diagrams of equipment or specimen curves are included. In fact the book contains no illustrations whatever, and one regrets the lack of these in certain sections.

In addition to a detailed table of contents facing page 1, the book is provided with a good subject and author index.

The reviewer feels that Dr. Pelshenke has contributed a useful manual, replete with carefully selected and tested methods. There has been a singular lack of such books of methods in the literature of recent years and this manual should be of large service in the laboratories engaged in cereal testing and research.

C. H. Bailey